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(54) Title: RESPIRATORY SYNCYTIAL VIRUS (RSV) INFECTION

(57) Abstract: The invention describes methods of treating and preventing respiratory syncytial virus (RSV) infection comprising administering a TNF inhibitor and an additional therapeutic agent. The invention also describes methods of treating and preventing respiratory syncytial virus (RSV) infection comprising administering an anti-TNF antibody and an anti-RSV antibody.



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TREATMENT OF  
RESPIRATORY SYNCYTIAL VIRUS (RSV) INFECTION

RELATED APPLICATIONS

5           This application claims priority to U.S. Provisional Application No. 60/617563, filed October 8, 2004. This application is related to U.S. Patent Nos. 6,090,382, 6,258,562, and 6,509,015. This application is also related to U.S. Patent Application Serial No. 09/801,185, filed March 7, 2001; U.S. Patent Application Serial No. 10/302,356, filed November 22, 2002; U.S. Patent Application Serial No. 10/163657, 10 filed June 5, 2002; and U.S. Patent Application Serial No. 10/133715, filed April 26, 2002; U.S. Patent Application Serial No. 10/222140, filed August 16, 2002; U.S. Patent Application Serial No. 10/693233, filed October 24, 2003; U.S. Patent Application Serial No. 10/622932, filed July 18, 2003; U.S. Patent Application Serial No. 10/623039, filed July 18, 2003; U.S. Patent Application Serial No. 10/623076, filed July 15 18, 2003; U.S. Patent Application Serial No. 10/623065, filed July 18, 2003; U.S. Patent Application Serial No. 10/622928, filed July 18, 2003; U.S. Patent Application Serial No. 10/623075, filed July 18, 2003; U.S. Patent Application Serial No. 10/623035, filed July 18, 2003; U.S. Patent Application Serial No. 10/622683, filed July 18, 2003; U.S. Patent Application Serial No. 10/622205, filed July 18, 2003; U.S. Patent Application 20 Serial No. 10/622210, filed July 18, 2003; and U.S. Patent Application Serial No. 10/623318, filed July 18, 2003. This application is also related to PCT/US2005/012007 and US Appln. No. 11/104117. The entire contents of each of these patents and patent applications are hereby incorporated herein by reference.

25   BACKGROUND OF THE INVENTION

          Cytokines, such as interleukin-1 (IL-1) and tumor necrosis factor (TNF) are molecules produced by a variety of cells, such as monocytes and macrophages, which have been identified as mediators of inflammatory processes. Cytokines, including TNF, regulate the intensity and duration of the inflammatory response which occurs as the 30 result of an injury or infection. Elevated levels of TNF play an important role in pathologic inflammation. TNF also referred to as (TNF $\alpha$ ) has been implicated in the pathophysiology of a variety of human diseases and disorders, including sepsis, infections, autoimmune diseases, transplant rejection and graft-versus-host disease (see *e.g.*, Moeller *et al.* (1990) *Cytokine* 2:162; U.S. Patent No. 5,231,024 to Moeller *et al.*; 35 European Patent Publication No. 260 610 B1 by Moeller, A. *et al.*; Vasilli (1992) *Annu. Rev. Immunol.* 10:411; Tracey and Cerami (1994) *Annu. Rev. Med.* 45:491).

Cytokines, including TNF, have also been implicated in the pathophysiology of respiratory syncytial virus (RSV) infection (Franke *et al.* (1995) *Adv Exp Med Biol.* 371B:785 and Carpenter *et al.* (2002) *BMC Infect Dis.* 2:5). RSV is a pneumovirus that is responsible for the majority of respiratory illnesses and deaths in young children, as well as the elderly (Glezen *et al.* (1973) *N. Engl. J. Med.* 288:498; Shay *et al.* (1999) *J. Am. Med. Assoc.* 282:1440). About 1% of primary RSV infections result in hospitalization (Baker and Ryan (1999) *Postgrad Med.* 106:97). Today treatment often includes supplemental oxygen and medications which provide respiratory support. There remains a need to engineer safe and effective vaccines that will alleviate the serious health problems attributable to RSV, as early efforts at a vaccine failed, as the vaccines caused severe illness and some mortality (Kim *et al.* (1969) *Am. J. Epidemiol.* 89:442).

### SUMMARY OF THE INVENTION

There is a need to treat and prevent respiratory syncytial virus (RSV) infection and disorders associated with RSV infection in a safe and effective manner. The present invention includes methods of treatment and prevention of RSV infection comprising administering TNF inhibitors, including anti-TNF antibodies.

The invention includes a method for treating a human subject suffering from respiratory syncytial virus (RSV) infection, comprising administering to the subject an anti-TNF $\alpha$  antibody and an additional therapeutic agent, such that the RSV infection is treated. In one embodiment, the anti-TNF $\alpha$  antibody is a human antibody.

The invention describes a method for treating a human subject suffering from RSV infection, comprising administering to the subject an anti-TNF $\alpha$  antibody and an additional therapeutic agent, such that the RSV infection is treated, wherein the antibody is an isolated human antibody, or an antigen-binding portion thereof, that dissociates from human TNF $\alpha$  with a  $K_d$  of  $1 \times 10^{-8}$  M or less and a  $K_{off}$  rate constant of  $1 \times 10^{-3} \text{ s}^{-1}$  or less, both determined by surface plasmon resonance, and neutralizes human TNF $\alpha$  cytotoxicity in a standard *in vitro* L929 assay with an  $IC_{50}$  of  $1 \times 10^{-7}$  M or less.

The invention also describes a method for treating a human subject suffering from RSV infection, comprising administering to the subject an anti-TNF $\alpha$  antibody and an additional therapeutic agent, such that the RSV infection is treated, wherein the antibody is an isolated human antibody, or antigen-binding portion thereof, with the following characteristics:

a) dissociates from human TNF $\alpha$  with a  $K_{off}$  rate constant of  $1 \times 10^{-3} \text{ s}^{-1}$  or less, as determined by surface plasmon resonance;

b) has a light chain CDR3 domain comprising the amino acid sequence of SEQ ID NO: 3, or modified from SEQ ID NO: 3 by a single alanine substitution at position 1, 4, 5, 7 or 8 or by one to five conservative amino acid substitutions at positions 1, 3, 4, 6, 7, 8 and/or 9;

- 5 c) has a heavy chain CDR3 domain comprising the amino acid sequence of SEQ ID NO: 4, or modified from SEQ ID NO: 4 by a single alanine substitution at position 2, 3, 4, 5, 6, 8, 9, 10 or 11 or by one to five conservative amino acid substitutions at positions 2, 3, 4, 5, 6, 8, 9, 10, 11 and/or 12.

The invention also pertains to a method for treating a human subject suffering from RSV infection, comprising administering to the subject an anti-TNF $\alpha$  antibody and an additional therapeutic agent, such that the RSV infection is treated,, wherein the antibody is an isolated human antibody, or an antigen binding portion thereof, with a light chain variable region (LCVR) comprising the amino acid sequence of SEQ ID NO: 1 and a heavy chain variable region (HCVR) comprising the amino acid sequence of

15 SEQ ID NO: 2

The invention includes a method for treating a human subject suffering from RSV infection, comprising administering to the subject an anti-TNF $\alpha$  antibody and an additional therapeutic agent, wherein the antibody is D2E7.

In one embodiment of the invention, the additional therapeutic agent is selected from the group consisting of adrenaline, a bronchodilator drug, a corticosteroid, ribavirin, a leukotriene antagonist, epinephrine, an antibiotic, supplemental oxygen, and an anti-RSV antibody. In another embodiment, the subject is using mechanical ventilation.

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The invention also includes a method for preventing an RSV-associated disorder in a human subject, comprising administering to the subject an anti-TNF $\alpha$  antibody and an additional therapeutic agent. In one embodiment, the anti-TNF $\alpha$  antibody is a human antibody.

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The invention also describes a method for preventing an RSV-associated disorder in a human subject, comprising administering to the subject an anti-TNF $\alpha$  antibody and an additional therapeutic agent, wherein the antibody is an isolated human antibody, or an antigen-binding portion thereof, that dissociates from human TNF $\alpha$  with a  $K_d$  of  $1 \times 10^{-8}$  M or less and a  $K_{off}$  rate constant of  $1 \times 10^{-3} \text{ s}^{-1}$  or less, both determined by surface plasmon resonance, and neutralizes human TNF $\alpha$  cytotoxicity in a standard *in vitro* L929 assay with an  $IC_{50}$  of  $1 \times 10^{-7}$  M or less.

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The invention includes a method for preventing an RSV-associated disorder in a human subject, comprising administering to the subject an anti-TNF $\alpha$  antibody and an

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additional therapeutic agent, wherein the antibody is an isolated human antibody, or antigen-binding portion thereof, with the following characteristics:

- a) dissociates from human TNF $\alpha$  with a K<sub>off</sub> rate constant of  $1 \times 10^{-3} \text{ s}^{-1}$  or less, as determined by surface plasmon resonance;
- 5        b) has a light chain CDR3 domain comprising the amino acid sequence of SEQ ID NO: 3, or modified from SEQ ID NO: 3 by a single alanine substitution at position 1, 4, 5, 7 or 8 or by one to five conservative amino acid substitutions at positions 1, 3, 4, 6, 7, 8 and/or 9;
- 10       c) has a heavy chain CDR3 domain comprising the amino acid sequence of SEQ ID NO: 4, or modified from SEQ ID NO: 4 by a single alanine substitution at position 2, 3, 4, 5, 6, 8, 9, 10 or 11 or by one to five conservative amino acid substitutions at positions 2, 3, 4, 5, 6, 8, 9, 10, 11 and/or 12.

The invention provides a method for preventing an RSV-associated disorder in a human subject, comprising administering to the subject an anti-TNF $\alpha$  antibody and an  
15 additional therapeutic agent, wherein the antibody is an isolated human antibody, or an antigen binding portion thereof, with a light chain variable region (LCVR) comprising the amino acid sequence of SEQ ID NO: 1 and a heavy chain variable region (HCVR) comprising the amino acid sequence of SEQ ID NO: 2

The invention also provides a method for preventing an RSV-associated disorder  
20 in a human subject, comprising administering to the subject an anti-TNF $\alpha$  antibody and an additional therapeutic agent, wherein the antibody is D2E7.

In one embodiment of the invention, the additional therapeutic agent is an anti-RSV antibody. In an additional embodiment, the anti-RSV antibody is palivizumab (Synagis®). In a further embodiment, the anti-RSV antibody is a human RSV-IGIV  
25 antibody (RespiGam®) or motivizumab (Numax™).

The invention describes a method for treating RSV infection or preventing RSV-associated disorders in a human subject, comprising administering to the subject a combination treatment comprising a D2E7 antibody and a palivizumab antibody (Synagis). In one embodiment, the D2E7 antibody and the palivizumab antibody are co-  
30 formulated. In one embodiment of the invention, the subject is a child or an infant. In one embodiment the subject was born prematurely. In another embodiment the subject was born at less than 28 weeks of gestation. In another embodiment, the subject was born between 28 and 32 weeks of gestation. In yet another embodiment, the subject was born between 32 and 35 weeks of gestation. In another embodiment, the subject has  
35 chronic lung disease, such as bronchopulmonary dysplasia. In yet another embodiment,

the subject has congenital heart disease, such as hemodynamically significant congenital heart disease.

The invention also includes an immunoprophylactic method comprising administering an anti-RSV antibody to a subject at risk for RSV infection in combination  
5 with an anti-TNF antibody. The invention further describes a method of preventing RSV infection in a subject at high risk for RSV infection comprising administering an anti-RSV antibody and an anti-TNF antibody. In one embodiment, the anti-RSV antibody is selected from the group of motivizumab, human RSV-IGIV, and palivizumab. In one  
10 embodiment the anti-TNF antibody is D2E7 (adalimumab). In one embodiment the subject was born prematurely. In another embodiment the subject was born at less than 28 weeks of gestation. In another embodiment, the subject was born between 28 and 32 weeks of gestation. In yet another embodiment, the subject was born between 32 and 35 weeks of gestation. In another embodiment, the subject has chronic lung disease, such as bronchopulmonary dysplasia. In yet another embodiment, the subject has congenital  
15 heart disease, such as hemodynamically significant congenital heart disease.

In one embodiment, the RSV-associated disorder is a respiratory complication. In another embodiment, the RSV-associated disorder is selected from the group consisting of nasal congestion, nasal flaring, coughing, rapid breathing, breathing difficulty, fever, shortness of breath, wheezing, and hypoxia, pneumonia, bronchitis, and  
20 croup.

In one embodiment of the invention, the additional agent and the anti-TNF antibody are administered sequentially to a patient in need thereof. In another embodiment, an anti-RSV antibody and an anti-TNF antibody are administered sequentially to a patient in need thereof.

25 The invention describes a pharmaceutical composition comprising D2E7, palivizumab, and a pharmaceutically acceptable carrier.

The invention also describes a kit comprising: a pharmaceutical composition comprising an anti-TNF $\alpha$  antibody and a pharmaceutically acceptable carrier; at least one pharmaceutical composition each comprising an additional therapeutic agent and a  
30 pharmaceutically acceptable carrier; and instructions for administration of the pharmaceutical composition of (a) and (b) for the treatment of RSV infection or prevention of RSV-associated disorders. In one embodiment, the anti-TNF $\alpha$  antibody is D2E7.

The invention also provides a kit comprising: a pharmaceutical composition  
35 comprising D2E7 and a pharmaceutically acceptable carrier; a pharmaceutical composition comprising an anti-RSV antibody and a pharmaceutically acceptable

carrier; and instructions for administration of D2E7 and the anti-RSV antibody for the prevention of RSV-associated disorders. In one embodiment, the anti-RSV antibody is palivizumab (Synagis®). In another embodiment, the anti-RSV antibody is RespiGam® or Numax™ (motavizumab).

- 5           The invention also includes a formulation comprising D2E7 and palivizumab for the treatment of RSV infection or prevention of RSV-associated disorders. In one embodiment, the formulation is in liquid form.

## DETAILED DESCRIPTION OF THE INVENTION

10

### I. Definitions

In order that the present invention may be more readily understood, certain terms are first defined.

- 15           The term "human TNFα" (abbreviated herein as hTNFα, or simply hTNF), as used herein, is intended to refer to a human cytokine that exists as a 17 kD secreted form and a 26 kD membrane associated form, the biologically active form of which is composed of a trimer of noncovalently bound 17 kD molecules. The structure of hTNFα is described further in, for example, Pennica, D., *et al.* (1984) *Nature* 312:724-729;
- 20   Davis, J.M., *et al.* (1987) *Biochemistry* 26:1322-1326; and Jones, E.Y., *et al.* (1989) *Nature* 338:225-228. The term human TNFα is intended to include recombinant human TNFα (rhTNFα), which can be prepared by standard recombinant expression methods or purchased commercially (R & D Systems, Catalog No. 210-TA, Minneapolis, MN). TNFα is also referred to as TNF.

- 25           The term "TNFα inhibitor" includes agents which interfere with TNFα activity. Examples of TNFα inhibitors include etanercept (Enbrel®, Amgen), infliximab (Remicade®, Johnson and Johnson), human anti-TNF monoclonal antibody (D2E7/HUMIRA®, Abbott Laboratories), CDP 571 (Celltech), and CDP 870 (Celltech) and other compounds which inhibit TNFα activity, such that when administered to a
- 30   subject suffering from or at risk of suffering from a disorder in which TNFα activity is detrimental, the disorder is treated. The term also includes each of the anti-TNFα human antibodies and antibody portions described herein as well as those described in U.S. Patent Nos. 6,090,382; 6,258,562; 6,509,015, and in U.S. Patent Application Serial Nos. 09/801185 and 10/302356.

- 35           The term "antibody", as used herein, is intended to refer to immunoglobulin molecules comprised of four polypeptide chains, two heavy (H) chains and two light (L)

chains inter-connected by disulfide bonds. Each heavy chain is comprised of a heavy chain variable region (abbreviated herein as HCVR or VH) and a heavy chain constant region. The heavy chain constant region is comprised of three domains, CH1, CH2 and CH3. Each light chain is comprised of a light chain variable region (abbreviated herein as LCVR or VL) and a light chain constant region. The light chain constant region is comprised of one domain, CL. The VH and VL regions can be further subdivided into regions of hypervariability, termed complementarity determining regions (CDR), interspersed with regions that are more conserved, termed framework regions (FR). Each VH and VL is composed of three CDRs and four FRs, arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4. The antibodies of the invention are described in further detail in U.S. Patent Nos. 6,090,382; 6,258,562; and 6,509,015, and in U.S. Patent Application Serial Nos. 09/801185 and 10/302356, each of which is incorporated herein by reference in its entirety.

The term "antigen-binding portion" of an antibody (or simply "antibody portion"), as used herein, refers to one or more fragments of an antibody that retain the ability to specifically bind to an antigen (*e.g.*, hTNF $\alpha$ ). It has been shown that the antigen-binding function of an antibody can be performed by fragments of a full-length antibody. Examples of binding fragments encompassed within the term "antigen-binding portion" of an antibody include (i) a Fab fragment, a monovalent fragment consisting of the VL, VH, CL and CH1 domains; (ii) a F(ab')<sub>2</sub> fragment, a bivalent fragment comprising two Fab fragments linked by a disulfide bridge at the hinge region; (iii) a Fd fragment consisting of the VH and CH1 domains; (iv) a Fv fragment consisting of the VL and VH domains of a single arm of an antibody, (v) a dAb fragment (Ward *et al.*, (1989) *Nature* 341:544-546), which consists of a VH domain; and (vi) an isolated complementarity determining region (CDR). Furthermore, although the two domains of the Fv fragment, VL and VH, are coded for by separate genes, they can be joined, using recombinant methods, by a synthetic linker that enables them to be made as a single protein chain in which the VL and VH regions pair to form monovalent molecules (known as single chain Fv (scFv); see *e.g.*, Bird *et al.* (1988) *Science* 242:423-426; and Huston *et al.* (1988) *Proc. Natl. Acad. Sci. USA* 85:5879-5883). Such single chain antibodies are also intended to be encompassed within the term "antigen-binding portion" of an antibody. Other forms of single chain antibodies, such as diabodies are also encompassed. Diabodies are bivalent, bispecific antibodies in which VH and VL domains are expressed on a single polypeptide chain, but using a linker that is too short to allow for pairing between the two domains on the same chain, thereby forcing the



domains to pair with complementary domains of another chain and creating two antigen binding sites (see *e.g.*, Holliger, P., *et al.* (1993) *Proc. Natl. Acad. Sci. USA* 90:6444-6448; Poljak, R.J., *et al.* (1994) *Structure* 2:1121-1123). The antibody portions of the invention are described in further detail in U.S. Patent Nos. 6,090,382, 6,258,562, 5 6,509,015, and in U.S. Patent Application Serial Nos. 09/801185 and 10/302356, each of which is incorporated herein by reference in its entirety.

Binding fragments are produced by recombinant DNA techniques, or by enzymatic or chemical cleavage of intact immunoglobulins. Binding fragments include Fab, Fab', F(ab')<sub>2</sub>, Fabc, Fv, single chains, and single-chain antibodies. Other than 10 "bispecific" or "bifunctional" immunoglobulins or antibodies, an immunoglobulin or antibody is understood to have each of its binding sites identical. A "bispecific" or "bifunctional antibody" is an artificial hybrid antibody having two different heavy/light chain pairs and two different binding sites. Bispecific antibodies can be produced by a variety of methods including fusion of hybridomas or linking of Fab' fragments. See, 15 *e.g.*, Songsivilai & Lachmann, *Clin. Exp. Immunol.* 79:315-321 (1990); Kostelny *et al.*, *J. Immunol.* 148, 1547-1553 (1992).

A "conservative amino acid substitution", as used herein, is one in which one amino acid residue is replaced with another amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been defined in 20 the art, including basic side chains (*e.g.*, lysine, arginine, histidine), acidic side chains (*e.g.*, aspartic acid, glutamic acid), uncharged polar side chains (*e.g.*, glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (*e.g.*, alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (*e.g.*, threonine, valine, isoleucine) and aromatic side chains (*e.g.*, 25 tyrosine, phenylalanine, tryptophan, histidine).

The term "human antibody", as used herein, is intended to include antibodies having variable and constant regions derived from human germline immunoglobulin sequences. The human antibodies of the invention may include amino acid residues not encoded by human germline immunoglobulin sequences (*e.g.*, mutations introduced by 30 random or site-specific mutagenesis *in vitro* or by somatic mutation *in vivo*), for example in the CDRs and in particular CDR3. However, the term "human antibody", as used herein, is not intended to include antibodies in which CDR sequences derived from the germline of another mammalian species, such as a mouse, have been grafted onto human framework sequences.

35 The term "recombinant human antibody", as used herein, is intended to include all human antibodies that are prepared, expressed, created or isolated by recombinant

means, such as antibodies expressed using a recombinant expression vector transfected into a host cell (described further below), antibodies isolated from a recombinant, combinatorial human antibody library (described further below), antibodies isolated from an animal (*e.g.*, a mouse) that is transgenic for human immunoglobulin genes (see 5 *e.g.*, Taylor, L.D. *et al.* (1992) *Nucl. Acids Res.* 20:6287) or antibodies prepared, expressed, created or isolated by any other means that involves splicing of human immunoglobulin gene sequences to other DNA sequences. Such recombinant human antibodies have variable and constant regions derived from human germline immunoglobulin sequences. In certain embodiments, however, such recombinant human 10 antibodies are subjected to *in vitro* mutagenesis (or, when an animal transgenic for human Ig sequences is used, *in vivo* somatic mutagenesis) and thus the amino acid sequences of the VH and VL regions of the recombinant antibodies are sequences that, while derived from and related to human germline VH and VL sequences, may not naturally exist within the human antibody germline repertoire *in vivo*.

15 An "isolated antibody", as used herein, is intended to refer to an antibody that is substantially free of other antibodies having different antigenic specificities (*e.g.*, an isolated antibody that specifically binds hTNF $\alpha$  is substantially free of antibodies that specifically bind antigens other than hTNF $\alpha$ ). An isolated antibody that specifically binds hTNF $\alpha$  may, however, have cross-reactivity to other antigens, such as TNF $\alpha$  20 molecules from other species (discussed in further detail below). Moreover, an isolated antibody may be substantially free of other cellular material and/or chemicals.

A "neutralizing antibody", as used herein (or an "antibody that neutralized hTNF $\alpha$  activity"), is intended to refer to an antibody whose binding to hTNF $\alpha$  results in inhibition of the biological activity of hTNF $\alpha$ . This inhibition of the biological activity 25 of hTNF $\alpha$  can be assessed by measuring one or more indicators of hTNF $\alpha$  biological activity, such as hTNF $\alpha$ -induced cytotoxicity (either *in vitro* or *in vivo*), hTNF $\alpha$ -induced cellular activation and hTNF $\alpha$  binding to hTNF $\alpha$  receptors. These indicators of hTNF $\alpha$  biological activity can be assessed by one or more of several standard *in vitro* or *in vivo* assays known in the art (see U.S. Patent No. 6,090,382). Preferably, the ability of an 30 antibody to neutralize hTNF $\alpha$  activity is assessed by inhibition of hTNF $\alpha$ -induced cytotoxicity of L929 cells. As an additional or alternative parameter of hTNF $\alpha$  activity, the ability of an antibody to inhibit hTNF $\alpha$ -induced expression of ELAM-1 on HUVEC, as a measure of hTNF $\alpha$ -induced cellular activation, can be assessed.

The term "surface plasmon resonance", as used herein, refers to an optical 35 phenomenon that allows for the analysis of real-time biospecific interactions by detection of alterations in protein concentrations within a biosensor matrix, for example

using the BIAcore system (Pharmacia Biosensor AB, Uppsala, Sweden and Piscataway, NJ). For further descriptions, see Example 1 of U.S. Patent 6,258,562 and Jönsson *et al.* (1993) *Ann. Biol. Clin.* 51:19; Jönsson *et al.* (1991) *Biotechniques* 11:620-627; Johnsson *et al.* (1995) *J. Mol. Recognit.* 8:125; and Johnsson *et al.* (1991) *Anal. Biochem.* 198:268.

5       The term "K<sub>off</sub>", as used herein, is intended to refer to the off rate constant for dissociation of an antibody from the antibody/antigen complex.

      The term "K<sub>d</sub>", as used herein, is intended to refer to the dissociation constant of a particular antibody-antigen interaction.

10       The term "IC<sub>50</sub>" as used herein, is intended to refer to the concentration of the inhibitor required to inhibit the biological endpoint of interest, *e.g.*, neutralize cytotoxicity activity.

      The term "nucleic acid molecule", as used herein, is intended to include DNA molecules and RNA molecules. A nucleic acid molecule may be single-stranded or double-stranded, but preferably is double-stranded DNA.

15       The term "isolated nucleic acid molecule", as used herein in reference to nucleic acids encoding antibodies or antibody portions (*e.g.*, VH, VL, CDR3) that bind hTNF $\alpha$ , is intended to refer to a nucleic acid molecule in which the nucleotide sequences encoding the antibody or antibody portion are free of other nucleotide sequences encoding antibodies or antibody portions that bind antigens other than hTNF $\alpha$ , which  
20       other sequences may naturally flank the nucleic acid in human genomic DNA. Thus, for example, an isolated nucleic acid of the invention encoding a VH region of an anti-hTNF $\alpha$  antibody contains no other sequences encoding other VH regions that bind antigens other than hTNF $\alpha$ .

      The term "vector", as used herein, is intended to refer to a nucleic acid molecule  
25       capable of transporting another nucleic acid to which it has been linked. One type of vector is a "plasmid", which refers to a circular double stranded DNA loop into which additional DNA segments may be ligated. Another type of vector is a viral vector, wherein additional DNA segments may be ligated into the viral genome. Certain vectors are capable of autonomous replication in a host cell into which they are introduced (*e.g.*,  
30       bacterial vectors having a bacterial origin of replication and episomal mammalian vectors). Other vectors (*e.g.*, non-episomal mammalian vectors) can be integrated into the genome of a host cell upon introduction into the host cell, and thereby are replicated along with the host genome. Moreover, certain vectors are capable of directing the expression of genes to which they are operatively linked. Such vectors are referred to  
35       herein as "recombinant expression vectors" (or simply, "expression vectors"). In general, expression vectors of utility in recombinant DNA techniques are often in the

form of plasmids. In the present specification, "plasmid" and "vector" may be used interchangeably as the plasmid is the most commonly used form of vector. However, the invention is intended to include such other forms of expression vectors, such as viral vectors (*e.g.*, replication defective retroviruses, adenoviruses and adeno-associated viruses), which serve equivalent functions.

The term "recombinant host cell" (or simply "host cell"), as used herein, is intended to refer to a cell into which a recombinant expression vector has been introduced. It should be understood that such terms are intended to refer not only to the particular subject cell but to the progeny of such a cell. Because certain modifications may occur in succeeding generations due to either mutation or environmental influences, such progeny may not, in fact, be identical to the parent cell, but are still included within the scope of the term "host cell" as used herein.

The term "dose," as used herein, refers to an amount of TNF $\alpha$  inhibitor which is administered to a subject.

The term "multiple-variable dose" includes different doses of a TNF $\alpha$  inhibitor which are administered to a subject for therapeutic treatment. "Multiple-variable dose regimen" or "multiple-variable dose therapy" describe a treatment schedule which is based on administering different amounts of TNF $\alpha$  inhibitor at various time points throughout the course of treatment. In one embodiment, the invention describes a multiple-variable dose method of treatment comprising an induction phase and a treatment phase, wherein a TNF $\alpha$  inhibitor is administered at a higher dose during the induction phase than the treatment phase. Multiple-variable dose regimens are described in PCT/US2005/012007 and US Appln. No. 11/104117.

The term "induction phase" or "loading phase", as used herein, refers to a period of treatment comprising administration of a TNF $\alpha$  inhibitor to a subject in order to attain a threshold level. During the induction phase, at least one induction dose of TNF $\alpha$  inhibitor is administered to a subject suffering from a disorder in which TNF $\alpha$  is detrimental.

The term "threshold level", as used herein, refers to a therapeutically effective level of a TNF $\alpha$  inhibitor in a subject. A threshold level is achieved by administering at least one induction dose during the induction phase of treatment. Any number of induction doses may be administered to achieve a threshold level of TNF $\alpha$  inhibitor. Once a threshold level is achieved, the treatment phase is initiated.

The term "induction dose" or "loading dose," used interchangeably herein, refers to the first dose of TNF $\alpha$  inhibitor, which is larger in comparison to the maintenance or treatment dose. The induction dose can be a single dose or, alternatively, a set of doses.

The induction dose is often used to bring the drug in the body to a steady state amount, and may be used to which to achieve maintenance drug levels quickly. An induction dose is subsequently followed by administration of smaller doses of TNF $\alpha$  inhibitor, *i.e.*, the treatment dose. The induction dose is administered during the induction phase of therapy. In one embodiment of the invention, the induction dose is at least twice the given amount of the treatment dose. In another embodiment of the invention, the induction dose of D2E7 is about 160 mg. In another embodiment, the induction dose of D2E7 is about 80 mg.

The term "treatment phase" or "maintenance phase", as used herein, refers to a period of treatment comprising administration of a TNF $\alpha$  inhibitor to a subject in order to maintain a desired therapeutic effect. The treatment phase follows the induction phase, and, therefore, is initiated once a threshold level is achieved.

The term "treatment dose" or "maintenance dose" is the amount of TNF $\alpha$  inhibitor or taken by a subject to maintain or continue a desired therapeutic effect. A treatment dose is administered subsequent to the induction dose. A treatment dose can be a single dose or, alternatively, a set of doses. A treatment dose is administered during the treatment phase of therapy. Treatment doses are smaller than the induction dose and can be equal to each other when administered in succession. In one embodiment, the invention describes at least one induction dose of D2E7 of about 160 mg, followed by at least one treatment dose of about 80 mg. In another embodiment, the invention describes at least one induction dose of D2E7 of 80 mg, followed by at least one treatment dose of 40 mg. In still another embodiment, the treatment dose is administered at least two weeks following the induction dose.

A "dosage regimen" or "dosing regimen" includes a treatment regimen based on a determined set of doses. In one embodiment, the invention describes a dosage regimen for the treatment or prevention of RSV infection, wherein D2E7, in combination with an additional therapeutic agent, is first administered as an induction dose and then administered in treatment doses which are lower than that of the induction dose.

The term "dosing", as used herein, refers to the administration of a substance (*e.g.*, an anti-TNF $\alpha$  antibody) to achieve a therapeutic objective (*e.g.*, the treatment of a TNF $\alpha$ -associated disorder).

The terms "biweekly dosing regimen", "biweekly dosing", and "biweekly administration", as used herein, refer to the time course of administering a substance (*e.g.*, an anti-TNF $\alpha$  antibody) to a subject to achieve a therapeutic objective (*e.g.*, the treatment of a TNF $\alpha$ -associated disorder). The biweekly dosing regimen is not intended to include a weekly dosing regimen. Preferably, the substance is administered every 9-

19 days, more preferably, every 11-17 days, even more preferably, every 13-15 days, and most preferably, every 14 days. Examples of a biweekly dosing regimen are described in PCT publication WO 02/100330.

The term "combination" as in the phrase "a first agent in combination with a second agent" includes co-administration of a first agent and a second agent, which for example may be dissolved or intermixed in the same pharmaceutically acceptable carrier, or administration of a first agent, followed by the second agent, or administration of the second agent, followed by the first agent. The present invention, therefore, includes methods of combination therapeutic treatment and combination pharmaceutical compositions. In one embodiment, the invention provides a combination therapy for treating or preventing RSV infection or symptoms related thereto comprising administering an anti-TNF antibody and an anti-RSV antibody. In another embodiment, the combination therapy of the invention comprises administration of D2E7 and palivizumab (Synagis®). In one embodiment, an anti-TNF antibody is administered to a subject who has previously been administered a therapeutic agent, such as an anti-RSV antibody, for the prevention of RSV infection.

The term "combination therapy", as used herein, refers to the administration of two or more therapeutic substances, *e.g.*, an anti-TNF $\alpha$  antibody and another drug, such as palivizumab (Synagis®). The other drug(s) may be administered accompanying, prior to, or following the administration of an anti-TNF $\alpha$  antibody.

In one embodiment, the combination therapy of the invention is concomitant. The term "concomitant" as in the phrase "concomitant therapeutic treatment" includes administering an agent in the presence of a second agent. A concomitant therapeutic treatment method includes methods in which the first, second, third, or additional agents are co-administered. A concomitant therapeutic treatment method also includes methods in which the first or additional agents are administered in the presence of a second or additional agent, wherein the second or additional agents, for example, may have been previously administered. A concomitant therapeutic treatment method may be executed step-wise by different actors. For example, one actor may administer to a subject a first agent and a second actor may administer to the subject a second agent, and the administering steps may be executed at the same time, or nearly the same time, or at distant times, so long as the first agent (and additional agents) are after administration in the presence of the second agent (and additional agents). The actor and the subject may be the same entity (*e.g.*, human).

The term "prophylactic treatment" or "prophylactic therapy" refers to administration of a therapeutic agent for the prevention of a disorder. In one

embodiment, the prophylactic treatment of the invention is used to prevent RSV infection, which includes prevention of disorders associated with RSV infection. In addition, the methods and kits of the invention may be used for immunoprophylaxis, which is prevention of infection by immunization.

5           The term "TNF $\alpha$ -mediated condition" or "TNF $\alpha$ -related disorder" refers to a local and/or systemic physiological disorder where TNF $\alpha$  is a primary mediator leading to the manifestation of the disorder. In one embodiment of the invention, the TNF $\alpha$ -related disorder is RSV infection.

10           The term "kit" as used herein refers to a packaged product comprising components with which to administer the TNF $\alpha$  antibody of the invention for treatment and prevention of RSV infection and disorders associated with RSV infection. The kit preferably comprises a box or container that holds the components of the kit. The box or container is affixed with a label or a Food and Drug Administration approved protocol. The box or container holds components of the invention which are preferably contained  
15           within plastic, polyethylene, polypropylene, ethylene, or propylene vessels. The vessels can be capped-tubes or bottles. The kit can also include instructions for administering the TNF $\alpha$  antibody of the invention. In one embodiment the kit of the invention includes the formulation comprising the human antibody D2E7, as described in PCT/IB03/04502 and U.S. Appln. No. 10/222140.

20           Various aspects of the invention are described in further detail herein.

## II. TNF $\alpha$ Inhibitors of the Invention

25           This invention provides a method of treating or preventing RSV infection in which the administration of a TNF $\alpha$  inhibitor is beneficial. In one embodiment, these methods include administration of isolated human antibodies, or antigen-binding portions thereof, that bind to human TNF $\alpha$  with high affinity and a low off rate, and have a high neutralizing capacity. Preferably, the human antibodies of the invention are recombinant, neutralizing human anti-hTNF $\alpha$  antibodies. The most preferred  
30           recombinant, neutralizing antibody of the invention is referred to herein as D2E7, also referred to as HUMIRA<sup>®</sup> or adalimumab (the amino acid sequence of the D2E7 VL region is shown in SEQ ID NO: 1; the amino acid sequence of the D2E7 VH region is shown in SEQ ID NO: 2). The properties of D2E7 (HUMIRA<sup>®</sup>) have been described in Salfeld *et al.*, U.S. Patent Nos. 6,090,382, 6,258,562, and 6,509,015, which are each  
35           incorporated by reference herein. Other examples of TNF $\alpha$  inhibitors include chimeric and humanized murine anti-hTNF $\alpha$  antibodies which have undergone clinical testing for

treatment of rheumatoid arthritis (see *e.g.*, Elliott, M.J., *et al.* (1994) *Lancet* 344:1125-1127; Elliot, M.J., *et al.* (1994) *Lancet* 344:1105-1110; Rankin, E.C., *et al.* (1995) *Br. J. Rheumatol.* 34:334-342).

In one embodiment, the method of treating or preventing RSV infection of the invention includes the administration of D2E7 antibodies and antibody portions, D2E7-related antibodies and antibody portions, and other human antibodies and antibody portions with equivalent properties to D2E7, such as high affinity binding to hTNF $\alpha$  with low dissociation kinetics and high neutralizing capacity. In one embodiment, the invention provides multiple-variable dose treatment with an isolated human antibody, or an antigen-binding portion thereof, that dissociates from human TNF $\alpha$  with a  $K_d$  of  $1 \times 10^{-8}$  M or less and a  $K_{off}$  rate constant of  $1 \times 10^{-3} \text{ s}^{-1}$  or less, both determined by surface plasmon resonance, and neutralizes human TNF $\alpha$  cytotoxicity in a standard *in vitro* L929 assay with an  $IC_{50}$  of  $1 \times 10^{-7}$  M or less. More preferably, the isolated human antibody, or antigen-binding portion thereof, dissociates from human TNF $\alpha$  with a  $K_{off}$  of  $5 \times 10^{-4} \text{ s}^{-1}$  or less, or even more preferably, with a  $K_{off}$  of  $1 \times 10^{-4} \text{ s}^{-1}$  or less. More preferably, the isolated human antibody, or antigen-binding portion thereof, neutralizes human TNF $\alpha$  cytotoxicity in a standard *in vitro* L929 assay with an  $IC_{50}$  of  $1 \times 10^{-8}$  M or less, even more preferably with an  $IC_{50}$  of  $1 \times 10^{-9}$  M or less and still more preferably with an  $IC_{50}$  of  $1 \times 10^{-10}$  M or less. In a preferred embodiment, the antibody is an isolated human recombinant antibody, or an antigen-binding portion thereof.

It is well known in the art that antibody heavy and light chain CDR3 domains play an important role in the binding specificity/affinity of an antibody for an antigen. Accordingly, in another aspect, the invention pertains to multiple-variable dose methods of treating a TNF $\alpha$ -related disorder in which the TNF $\alpha$  activity is detrimental by administering human antibodies that have slow dissociation kinetics for association with hTNF $\alpha$  and that have light and heavy chain CDR3 domains that structurally are identical to or related to those of D2E7. Position 9 of the D2E7 VL CDR3 can be occupied by Ala or Thr without substantially affecting the  $K_{off}$ . Accordingly, a consensus motif for the D2E7 VL CDR3 comprises the amino acid sequence: Q-R-Y-N-R-A-P-Y-(T/A) (SEQ ID NO: 3). Additionally, position 12 of the D2E7 VH CDR3 can be occupied by Tyr or Asn, without substantially affecting the  $K_{off}$ . Accordingly, a consensus motif for the D2E7 VH CDR3 comprises the amino acid sequence: V-S-Y-L-S-T-A-S-S-L-D-(Y/N) (SEQ ID NO: 4). Moreover, as demonstrated in Example 2 of U.S. Patent No. 6,090,382, the CDR3 domain of the D2E7 heavy and light chains is amenable to substitution with a single alanine residue (at position 1, 4, 5, 7 or 8 within the VL CDR3 or at position 2, 3, 4, 5, 6, 8, 9, 10 or 11 within the VH CDR3) without substantially



affecting the  $K_{\text{off}}$ . Still further, the skilled artisan will appreciate that, given the amenability of the D2E7 VL and VH CDR3 domains to substitutions by alanine, substitution of other amino acids within the CDR3 domains may be possible while still retaining the low off rate constant of the antibody, in particular substitutions with conservative amino acids. Preferably, no more than one to five conservative amino acid substitutions are made within the D2E7 VL and/or VH CDR3 domains. More preferably, no more than one to three conservative amino acid substitutions are made within the D2E7 VL and/or VH CDR3 domains. Additionally, conservative amino acid substitutions should not be made at amino acid positions critical for binding to hTNF $\alpha$ . Positions 2 and 5 of the D2E7 VL CDR3 and positions 1 and 7 of the D2E7 VH CDR3 appear to be critical for interaction with hTNF $\alpha$  and thus, conservative amino acid substitutions preferably are not made at these positions (although an alanine substitution at position 5 of the D2E7 VL CDR3 is acceptable, as described above) (*see* U.S. Patent No. 6,090,382).

Accordingly, in another embodiment, the invention provides methods of treating or preventing RSV infection by the administration of an isolated human antibody, or antigen-binding portion thereof. The antibody or antigen-binding portion thereof preferably contains the following characteristics:

- a) dissociates from human TNF $\alpha$  with a  $K_{\text{off}}$  rate constant of  $1 \times 10^{-3} \text{ s}^{-1}$  or less, as determined by surface plasmon resonance;
- b) has a light chain CDR3 domain comprising the amino acid sequence of SEQ ID NO: 3, or modified from SEQ ID NO: 3 by a single alanine substitution at position 1, 4, 5, 7 or 8 or by one to five conservative amino acid substitutions at positions 1, 3, 4, 6, 7, 8 and/or 9;
- c) has a heavy chain CDR3 domain comprising the amino acid sequence of SEQ ID NO: 4, or modified from SEQ ID NO: 4 by a single alanine substitution at position 2, 3, 4, 5, 6, 8, 9, 10 or 11 or by one to five conservative amino acid substitutions at positions 2, 3, 4, 5, 6, 8, 9, 10, 11 and/or 12.

More preferably, the antibody, or antigen-binding portion thereof, dissociates from human TNF $\alpha$  with a  $K_{\text{off}}$  of  $5 \times 10^{-4} \text{ s}^{-1}$  or less. Even more preferably, the antibody, or antigen-binding portion thereof, dissociates from human TNF $\alpha$  with a  $K_{\text{off}}$  of  $1 \times 10^{-4} \text{ s}^{-1}$  or less.

In yet another embodiment, the invention provides methods of treating or preventing RSV infection by the administration of an isolated human antibody, or antigen-binding portion thereof. The antibody or antigen-binding portion thereof preferably contains a light chain variable region (LCVR) having a CDR3 domain

comprising the amino acid sequence of SEQ ID NO: 3, or modified from SEQ ID NO: 3 by a single alanine substitution at position 1, 4, 5, 7 or 8, and with a heavy chain variable region (HCVR) having a CDR3 domain comprising the amino acid sequence of SEQ ID NO: 4, or modified from SEQ ID NO: 4 by a single alanine substitution at position 2, 3, 4, 5, 6, 8, 9, 10 or 11. Preferably, the LCVR further has a CDR2 domain comprising the amino acid sequence of SEQ ID NO: 5 (*i.e.*, the D2E7 VL CDR2) and the HCVR further has a CDR2 domain comprising the amino acid sequence of SEQ ID NO: 6 (*i.e.*, the D2E7 VH CDR2). Even more preferably, the LCVR further has CDR1 domain comprising the amino acid sequence of SEQ ID NO: 7 (*i.e.*, the D2E7 VL CDR1) and the HCVR has a CDR1 domain comprising the amino acid sequence of SEQ ID NO: 8 (*i.e.*, the D2E7 VH CDR1). The framework regions for VL preferably are from the V<sub>K</sub>I human germline family, more preferably from the A20 human germline Vk gene and most preferably from the D2E7 VL framework sequences shown in Figures 1A and 1B of U.S. Patent No. 6,090,382. The framework regions for VH preferably are from the V<sub>H</sub>3 human germline family, more preferably from the DP-31 human germline VH gene and most preferably from the D2E7 VH framework sequences shown in Figures 2A and 2B of U.S. Patent No. 6,090,382.

Accordingly, in another embodiment, the invention provides methods of treating or preventing RSV infection by the administration of an isolated human antibody, or antigen-binding portion thereof. The antibody or antigen-binding portion thereof preferably contains a light chain variable region (LCVR) comprising the amino acid sequence of SEQ ID NO: 1 (*i.e.*, the D2E7 VL) and a heavy chain variable region (HCVR) comprising the amino acid sequence of SEQ ID NO: 2 (*i.e.*, the D2E7 VH). In certain embodiments, the antibody comprises a heavy chain constant region, such as an IgG1, IgG2, IgG3, IgG4, IgA, IgE, IgM or IgD constant region. Preferably, the heavy chain constant region is an IgG1 heavy chain constant region or an IgG4 heavy chain constant region. Furthermore, the antibody can comprise a light chain constant region, either a kappa light chain constant region or a lambda light chain constant region. Preferably, the antibody comprises a kappa light chain constant region. Alternatively, the antibody portion can be, for example, a Fab fragment or a single chain Fv fragment.

In still other embodiments, the invention methods of treating or preventing RSV infection in which the administration of an anti-TNF $\alpha$  antibody is beneficial administration of an isolated human antibody, or an antigen-binding portions thereof. The antibody or antigen-binding portion thereof preferably contains D2E7-related VL and VH CDR3 domains, for example, antibodies, or antigen-binding portions thereof, with a light chain variable region (LCVR) having a CDR3 domain comprising an amino

acid sequence selected from the group consisting of SEQ ID NO: 3, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25 and SEQ ID NO: 26  
5 or with a heavy chain variable region (HCVR) having a CDR3 domain comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 4, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 30, SEQ ID NO: 31, SEQ ID NO: 32, SEQ ID NO: 33, SEQ ID NO: 34 and SEQ ID NO: 35.

In another embodiment, the TNF $\alpha$  inhibitor of the invention is etanercept  
10 (described in WO 91/03553 and WO 09/406476), infliximab (described in U.S. Patent No. 5,656,272), CDP571 (a humanized monoclonal anti-TNF-alpha IgG4 antibody), CDP 870 (a humanized monoclonal anti-TNF-alpha antibody fragment), D2E7 (a human anti-TNF mAb), soluble TNF receptor Type I, or a pegylated soluble TNF receptor Type I (PEGs TNF-R1).

15 The TNF $\alpha$  antibody of the invention can be modified. In some embodiments, the TNF $\alpha$  antibody or antigen binding fragments thereof, is chemically modified to provide a desired effect. For example, pegylation of antibodies and antibody fragments of the invention may be carried out by any of the pegylation reactions known in the art, as described, for example, in the following references: *Focus on Growth Factors* 3:4-10  
20 (1992); EP 0 154 316; and EP 0 401 384 (each of which is incorporated by reference herein in its entirety). Preferably, the pegylation is carried out via an acylation reaction or an alkylation reaction with a reactive polyethylene glycol molecule (or an analogous reactive water-soluble polymer). A preferred water-soluble polymer for pegylation of the antibodies and antibody fragments of the invention is polyethylene glycol (PEG). As  
25 used herein, "polyethylene glycol" is meant to encompass any of the forms of PEG that have been used to derivatize other proteins, such as mono (Cl-ClO) alkoxy- or aryloxy-polyethylene glycol.

Methods for preparing pegylated antibodies and antibody fragments of the invention will generally comprise the steps of (a) reacting the antibody or antibody  
30 fragment with polyethylene glycol, such as a reactive ester or aldehyde derivative of PEG, under conditions whereby the antibody or antibody fragment becomes attached to one or more PEG groups, and (b) obtaining the reaction products. It will be apparent to one of ordinary skill in the art to select the optimal reaction conditions or the acylation reactions based on known parameters and the desired result.

35 Pegylated antibodies and antibody fragments may generally be used to treat TNF $\alpha$ -related disorders of the invention by administration of the TNF $\alpha$  antibodies and

antibody fragments described herein. Generally the pegylated antibodies and antibody fragments have increased half-life, as compared to the nonpegylated antibodies and antibody fragments. The pegylated antibodies and antibody fragments may be employed alone, together, or in combination with other pharmaceutical compositions.

5 In yet another embodiment of the invention, TNF $\alpha$  antibodies or fragments thereof can be altered wherein the constant region of the antibody is modified to reduce at least one constant region-mediated biological effector function relative to an unmodified antibody. To modify an antibody of the invention such that it exhibits reduced binding to the Fc receptor, the immunoglobulin constant region segment of the  
10 antibody can be mutated at particular regions necessary for Fc receptor (FcR) interactions (see *e.g.*, Canfield, S.M. and S.L. Morrison (1991) *J. Exp. Med.* 173:1483-1491; and Lund, J. *et al.* (1991) *J. of Immunol.* 147:2657-2662). Reduction in FcR binding ability of the antibody may also reduce other effector functions which rely on FcR interactions, such as opsonization and phagocytosis and antigen-dependent cellular  
15 cytotoxicity.

An antibody or antibody portion of the invention can be derivatized or linked to another functional molecule (*e.g.*, another peptide or protein). Accordingly, the antibodies and antibody portions of the invention are intended to include derivatized and otherwise modified forms of the human anti-hTNF $\alpha$  antibodies described herein,  
20 including immunoadhesion molecules. For example, an antibody or antibody portion of the invention can be functionally linked (by chemical coupling, genetic fusion, noncovalent association or otherwise) to one or more other molecular entities, such as another antibody (*e.g.*, a bispecific antibody or a diabody), a detectable agent, a cytotoxic agent, a pharmaceutical agent, and/or a protein or peptide that can mediate association of  
25 the antibody or antibody portion with another molecule (such as a streptavidin core region or a polyhistidine tag).

One type of derivatized antibody is produced by crosslinking two or more antibodies (of the same type or of different types, *e.g.*, to create bispecific antibodies). Suitable crosslinkers include those that are heterobifunctional, having two distinctly  
30 reactive groups separated by an appropriate spacer (*e.g.*, m-maleimidobenzoyl-N-hydroxysuccinimide ester) or homobifunctional (*e.g.*, disuccinimidyl suberate). Such linkers are available from Pierce Chemical Company, Rockford, IL.

Useful detectable agents with which an antibody or antibody portion of the invention may be derivatized include fluorescent compounds. Exemplary fluorescent  
35 detectable agents include fluorescein, fluorescein isothiocyanate, rhodamine, 5-dimethylamine-1-naphthalenesulfonyl chloride, phycoerythrin and the like. An antibody

may also be derivatized with detectable enzymes, such as alkaline phosphatase, horseradish peroxidase, glucose oxidase and the like. When an antibody is derivatized with a detectable enzyme, it is detected by adding additional reagents that the enzyme uses to produce a detectable reaction product. For example, when the detectable agent  
5 horseradish peroxidase is present, the addition of hydrogen peroxide and diaminobenzidine leads to a colored reaction product, which is detectable. An antibody may also be derivatized with biotin, and detected through indirect measurement of avidin or streptavidin binding.

An antibody, or antibody portion, of the invention can be prepared by  
10 recombinant expression of immunoglobulin light and heavy chain genes in a host cell. To express an antibody recombinantly, a host cell is transfected with one or more recombinant expression vectors carrying DNA fragments encoding the immunoglobulin light and heavy chains of the antibody such that the light and heavy chains are expressed in the host cell and, preferably, secreted into the medium in which the host cells are  
15 cultured, from which medium the antibodies can be recovered. Standard recombinant DNA methodologies are used to obtain antibody heavy and light chain genes, incorporate these genes into recombinant expression vectors and introduce the vectors into host cells, such as those described in Sambrook, Fritsch and Maniatis (eds), *Molecular Cloning; A Laboratory Manual, Second Edition*, Cold Spring Harbor, N.Y.,  
20 (1989), Ausubel, F.M. *et al.* (eds.) *Current Protocols in Molecular Biology*, Greene Publishing Associates, (1989) and in U.S. Patent No. 4,816,397 by Boss *et al.*

To express D2E7 or a D2E7-related antibody, DNA fragments encoding the light and heavy chain variable regions are first obtained. These DNAs can be obtained by amplification and modification of germline light and heavy chain variable sequences  
25 using the polymerase chain reaction (PCR). Germline DNA sequences for human heavy and light chain variable region genes are known in the art (see *e.g.*, the "Vbase" human germline sequence database; see also Kabat, E.A., *et al.* (1991) *Sequences of Proteins of Immunological Interest, Fifth Edition*, U.S. Department of Health and Human Services, NIH Publication No. 91-3242; Tomlinson, I.M., *et al.* (1992) "The Repertoire of Human  
30 Germline V<sub>H</sub> Sequences Reveals about Fifty Groups of V<sub>H</sub> Segments with Different Hypervariable Loops" *J. Mol. Biol.* 227:776-798; and Cox, J.P.L. *et al.* (1994) "A Directory of Human Germ-line V<sub>H</sub> Segments Reveals a Strong Bias in their Usage" *Eur. J. Immunol.* 24:827-836; the contents of each of which are expressly incorporated herein by reference). To obtain a DNA fragment encoding the heavy chain variable  
35 region of D2E7, or a D2E7-related antibody, a member of the V<sub>H</sub>3 family of human germline V<sub>H</sub> genes is amplified by standard PCR. Most preferably, the DP-31 V<sub>H</sub>

germline sequence is amplified. To obtain a DNA fragment encoding the light chain variable region of D2E7, or a D2E7-related antibody, a member of the V<sub>κ</sub>I family of human germline VL genes is amplified by standard PCR. Most preferably, the A20 VL germline sequence is amplified. PCR primers suitable for use in amplifying the DP-31  
5 germline VH and A20 germline VL sequences can be designed based on the nucleotide sequences disclosed in the references cited *supra*, using standard methods.

Once the germline VH and VL fragments are obtained, these sequences can be mutated to encode the D2E7 or D2E7-related amino acid sequences disclosed herein. The amino acid sequences encoded by the germline VH and VL DNA sequences are first  
10 compared to the D2E7 or D2E7-related VH and VL amino acid sequences to identify amino acid residues in the D2E7 or D2E7-related sequence that differ from germline. Then, the appropriate nucleotides of the germline DNA sequences are mutated such that the mutated germline sequence encodes the D2E7 or D2E7-related amino acid sequence, using the genetic code to determine which nucleotide changes should be made.  
15 Mutagenesis of the germline sequences is carried out by standard methods, such as PCR-mediated mutagenesis (in which the mutated nucleotides are incorporated into the PCR primers such that the PCR product contains the mutations) or site-directed mutagenesis.

Once DNA fragments encoding D2E7 or D2E7-related VH and VL segments are obtained (by amplification and mutagenesis of germline VH and VL genes, as described  
20 above), these DNA fragments can be further manipulated by standard recombinant DNA techniques, for example to convert the variable region genes to full-length antibody chain genes, to Fab fragment genes or to a scFv gene. In these manipulations, a VL- or VH-encoding DNA fragment is operatively linked to another DNA fragment encoding another protein, such as an antibody constant region or a flexible linker. The term  
25 "operatively linked", as used in this context, is intended to mean that the two DNA fragments are joined such that the amino acid sequences encoded by the two DNA fragments remain in-frame.

The isolated DNA encoding the VH region can be converted to a full-length heavy chain gene by operatively linking the VH-encoding DNA to another DNA  
30 molecule encoding heavy chain constant regions (CH1, CH2 and CH3). The sequences of human heavy chain constant region genes are known in the art (see *e.g.*, Kabat, E.A., *et al.* (1991) *Sequences of Proteins of Immunological Interest, Fifth Edition*, U.S. Department of Health and Human Services, NIH Publication No. 91-3242) and DNA fragments encompassing these regions can be obtained by standard PCR amplification.  
35 The heavy chain constant region can be an IgG1, IgG2, IgG3, IgG4, IgA, IgE, IgM or IgD constant region, but most preferably is an IgG1 or IgG4 constant region. For a Fab

fragment heavy chain gene, the VH-encoding DNA can be operatively linked to another DNA molecule encoding only the heavy chain CH1 constant region.

The isolated DNA encoding the VL region can be converted to a full-length light chain gene (as well as a Fab light chain gene) by operatively linking the VL-encoding  
5 DNA to another DNA molecule encoding the light chain constant region, CL. The sequences of human light chain constant region genes are known in the art (see *e.g.*, Kabat, E.A., *et al.* (1991) *Sequences of Proteins of Immunological Interest, Fifth Edition*, U.S. Department of Health and Human Services, NIH Publication No. 91-3242) and DNA fragments encompassing these regions can be obtained by standard PCR  
10 amplification. The light chain constant region can be a kappa or lambda constant region, but most preferably is a kappa constant region.

To create a scFv gene, the VH- and VL-encoding DNA fragments are operatively linked to another fragment encoding a flexible linker, *e.g.*, encoding the amino acid sequence (Gly<sub>4</sub>-Ser)<sub>3</sub>, such that the VH and VL sequences can be expressed as a  
15 contiguous single-chain protein, with the VL and VH regions joined by the flexible linker (see *e.g.*, Bird *et al.* (1988) *Science* 242:423-426; Huston *et al.* (1988) *Proc. Natl. Acad. Sci. USA* 85:5879-5883; McCafferty *et al.*, *Nature* (1990) 348:552-554).

To express the antibodies, or antibody portions of the invention, DNAs encoding partial or full-length light and heavy chains, obtained as described above, are inserted  
20 into expression vectors such that the genes are operatively linked to transcriptional and translational control sequences. In this context, the term "operatively linked" is intended to mean that an antibody gene is ligated into a vector such that transcriptional and translational control sequences within the vector serve their intended function of regulating the transcription and translation of the antibody gene. The expression vector  
25 and expression control sequences are chosen to be compatible with the expression host cell used. The antibody light chain gene and the antibody heavy chain gene can be inserted into separate vector or, more typically, both genes are inserted into the same expression vector. The antibody genes are inserted into the expression vector by standard methods (*e.g.*, ligation of complementary restriction sites on the antibody gene  
30 fragment and vector, or blunt end ligation if no restriction sites are present). Prior to insertion of the D2E7 or D2E7-related light or heavy chain sequences, the expression vector may already carry antibody constant region sequences. For example, one approach to converting the D2E7 or D2E7-related VH and VL sequences to full-length antibody genes is to insert them into expression vectors already encoding heavy chain  
35 constant and light chain constant regions, respectively, such that the VH segment is operatively linked to the CH segment(s) within the vector and the VL segment is

operatively linked to the CL segment within the vector. Additionally or alternatively, the recombinant expression vector can encode a signal peptide that facilitates secretion of the antibody chain from a host cell. The antibody chain gene can be cloned into the vector such that the signal peptide is linked in-frame to the amino terminus of the antibody chain gene. The signal peptide can be an immunoglobulin signal peptide or a heterologous signal peptide (*i.e.*, a signal peptide from a non-immunoglobulin protein).

In addition to the antibody chain genes, the recombinant expression vectors of the invention carry regulatory sequences that control the expression of the antibody chain genes in a host cell. The term "regulatory sequence" is intended to include promoters, enhancers and other expression control elements (*e.g.*, polyadenylation signals) that control the transcription or translation of the antibody chain genes. Such regulatory sequences are described, for example, in Goeddel; *Gene Expression Technology: Methods in Enzymology* 185, Academic Press, San Diego, CA (1990). It will be appreciated by those skilled in the art that the design of the expression vector, including the selection of regulatory sequences may depend on such factors as the choice of the host cell to be transformed, the level of expression of protein desired, *etc.* Preferred regulatory sequences for mammalian host cell expression include viral elements that direct high levels of protein expression in mammalian cells, such as promoters and/or enhancers derived from cytomegalovirus (CMV) (such as the CMV promoter/enhancer), Simian Virus 40 (SV40) (such as the SV40 promoter/enhancer), adenovirus, (*e.g.*, the adenovirus major late promoter (AdMLP)) and polyoma. For further description of viral regulatory elements, and sequences thereof, see *e.g.*, U.S. Patent No. 5,168,062 by Stinski, U.S. Patent No. 4,510,245 by Bell *et al.* and U.S. Patent No. 4,968,615 by Schaffner *et al.*

In addition to the antibody chain genes and regulatory sequences, the recombinant expression vectors of the invention may carry additional sequences, such as sequences that regulate replication of the vector in host cells (*e.g.*, origins of replication) and selectable marker genes. The selectable marker gene facilitates selection of host cells into which the vector has been introduced (see *e.g.*, U.S. Patents Nos. 4,399,216, 4,634,665 and 5,179,017, all by Axel *et al.*). For example, typically the selectable marker gene confers resistance to drugs, such as G418, hygromycin or methotrexate, on a host cell into which the vector has been introduced. Preferred selectable marker genes include the dihydrofolate reductase (DHFR) gene (for use in *dhfr* host cells with methotrexate selection/amplification) and the *neo* gene (for G418 selection).

For expression of the light and heavy chains, the expression vector(s) encoding the heavy and light chains is transfected into a host cell by standard techniques. The



various forms of the term "transfection" are intended to encompass a wide variety of techniques commonly used for the introduction of exogenous DNA into a prokaryotic or eukaryotic host cell, *e.g.*, electroporation, calcium-phosphate precipitation, DEAE-dextran transfection and the like. Although it is theoretically possible to express the antibodies of the invention in either prokaryotic or eukaryotic host cells, expression of antibodies in eukaryotic cells, and most preferably mammalian host cells, is the most preferred because such eukaryotic cells, and in particular mammalian cells, are more likely than prokaryotic cells to assemble and secrete a properly folded and immunologically active antibody. Prokaryotic expression of antibody genes has been reported to be ineffective for production of high yields of active antibody (Boss, M.A. and Wood, C. R. (1985) *Immunology Today* 6:12-13).

Preferred mammalian host cells for expressing the recombinant antibodies of the invention include Chinese Hamster Ovary (CHO cells) (including dhfr- CHO cells, described in Urlaub and Chasin, (1980) *Proc. Natl. Acad. Sci. USA* 77:4216-4220, used with a DHFR selectable marker, *e.g.*, as described in R.J. Kaufman and P.A. Sharp (1982) *Mol. Biol.* 159:601-621), NS0 myeloma cells, COS cells and SP2 cells. When recombinant expression vectors encoding antibody genes are introduced into mammalian host cells, the antibodies are produced by culturing the host cells for a period of time sufficient to allow for expression of the antibody in the host cells or, more preferably, secretion of the antibody into the culture medium in which the host cells are grown. Antibodies can be recovered from the culture medium using standard protein purification methods.

Host cells can also be used to produce portions of intact antibodies, such as Fab fragments or scFv molecules. It is understood that variations on the above procedure are within the scope of the present invention. For example, it may be desirable to transfect a host cell with DNA encoding either the light chain or the heavy chain (but not both) of an antibody of this invention. Recombinant DNA technology may also be used to remove some or all of the DNA encoding either or both of the light and heavy chains that is not necessary for binding to hTNF $\alpha$ . The molecules expressed from such truncated DNA molecules are also encompassed by the antibodies of the invention. In addition, bifunctional antibodies may be produced in which one heavy and one light chain are an antibody of the invention and the other heavy and light chain are specific for an antigen other than hTNF $\alpha$  by crosslinking an antibody of the invention to a second antibody by standard chemical crosslinking methods.

In a preferred system for recombinant expression of an antibody, or antigen-binding portion thereof, of the invention, a recombinant expression vector encoding both

the antibody heavy chain and the antibody light chain is introduced into dhfr-CHO cells by calcium phosphate-mediated transfection. Within the recombinant expression vector, the antibody heavy and light chain genes are each operatively linked to CMV enhancer/AdMLP promoter regulatory elements to drive high levels of transcription of the genes. The recombinant expression vector also carries a DHFR gene, which allows for selection of CHO cells that have been transfected with the vector using methotrexate selection/amplification. The selected transformant host cells are culture to allow for expression of the antibody heavy and light chains and intact antibody is recovered from the culture medium. Standard molecular biology techniques are used to prepare the recombinant expression vector, transfect the host cells, select for transformants, culture the host cells and recover the antibody from the culture medium.

Recombinant human antibodies of the invention in addition to D2E7 or an antigen binding portion thereof, or D2E7-related antibodies disclosed herein can be isolated by screening of a recombinant combinatorial antibody library, preferably a scFv phage display library, prepared using human VL and VH cDNAs prepared from mRNA derived from human lymphocytes. Methodologies for preparing and screening such libraries are known in the art. In addition to commercially available kits for generating phage display libraries (*e.g.*, the Pharmacia *Recombinant Phage Antibody System*, catalog no. 27-9400-01; and the Stratagene *SurfZAP*<sup>TM</sup> phage display kit, catalog no. 240612), examples of methods and reagents particularly amenable for use in generating and screening antibody display libraries can be found in, for example, Ladner *et al.* U.S. Patent No. 5,223,409; Kang *et al.* PCT Publication No. WO 92/18619; Dower *et al.* PCT Publication No. WO 91/17271; Winter *et al.* PCT Publication No. WO 92/20791; Markland *et al.* PCT Publication No. WO 92/15679; Breitling *et al.* PCT Publication No. WO 93/01288; McCafferty *et al.* PCT Publication No. WO 92/01047; Garrard *et al.* PCT Publication No. WO 92/09690; Fuchs *et al.* (1991) *Bio/Technology* 9:1370-1372; Hay *et al.* (1992) *Hum Antibod Hybridomas* 3:81-85; Huse *et al.* (1989) *Science* 246:1275-1281; McCafferty *et al.*, *Nature* (1990) 348:552-554; Griffiths *et al.* (1993) *EMBO J* 12:725-734; Hawkins *et al.* (1992) *J Mol Biol* 226:889-896; Clackson *et al.* (1991) *Nature* 352:624-628; Gram *et al.* (1992) *PNAS* 89:3576-3580; Garrard *et al.* (1991) *Bio/Technology* 9:1373-1377; Hoogenboom *et al.* (1991) *Nuc Acid Res* 19:4133-4137; and Barbas *et al.* (1991) *PNAS* 88:7978-7982.

In a preferred embodiment, to isolate human antibodies with high affinity and a low off rate constant for hTNF $\alpha$ , a murine anti-hTNF $\alpha$  antibody having high affinity and a low off rate constant for hTNF $\alpha$  (*e.g.*, MAK 195, the hybridoma for which has deposit number ECACC 87 050801) is first used to select human heavy and light chain

sequences having similar binding activity toward hTNF $\alpha$ , using the epitope imprinting methods described in Hoogenboom *et al.*, PCT Publication No. WO 93/06213. The antibody libraries used in this method are preferably scFv libraries prepared and screened as described in McCafferty *et al.*, PCT Publication No. WO 92/01047, McCafferty *et al.*,  
5 *Nature* (1990) 348:552-554; and Griffiths *et al.*, (1993) *EMBO J* 12:725-734. The scFv antibody libraries preferably are screened using recombinant human TNF $\alpha$  as the antigen.

Once initial human VL and VH segments are selected, "mix and match" experiments, in which different pairs of the initially selected VL and VH segments are  
10 screened for hTNF $\alpha$  binding, are performed to select preferred VL/VH pair combinations. Additionally, to further improve the affinity and/or lower the off rate constant for hTNF $\alpha$  binding, the VL and VH segments of the preferred VL/VH pair(s) can be randomly mutated, preferably within the CDR3 region of VH and/or VL, in a process analogous to the *in vivo* somatic mutation process responsible for affinity  
15 maturation of antibodies during a natural immune response. This *in vitro* affinity maturation can be accomplished by amplifying VH and VL regions using PCR primers complimentary to the VH CDR3 or VL CDR3, respectively, which primers have been "spiked" with a random mixture of the four nucleotide bases at certain positions such that the resultant PCR products encode VH and VL segments into which random  
20 mutations have been introduced into the VH and/or VL CDR3 regions. These randomly mutated VH and VL segments can be rescreened for binding to hTNF $\alpha$  and sequences that exhibit high affinity and a low off rate for hTNF $\alpha$  binding can be selected.

Following screening and isolation of an anti-hTNF $\alpha$  antibody of the invention from a recombinant immunoglobulin display library, nucleic acid encoding the selected  
25 antibody can be recovered from the display package (*e.g.*, from the phage genome) and subcloned into other expression vectors by standard recombinant DNA techniques. If desired, the nucleic acid can be further manipulated to create other antibody forms of the invention (*e.g.*, linked to nucleic acid encoding additional immunoglobulin domains, such as additional constant regions). To express a recombinant human antibody isolated  
30 by screening of a combinatorial library, the DNA encoding the antibody is cloned into a recombinant expression vector and introduced into a mammalian host cells, as described in further detail in above.

Methods of isolating human antibodies with high affinity and a low off rate constant for hTNF $\alpha$  are also described in U.S. Patent Nos. 6,090,382, 6,258,562, and  
35 6,509,015, each of which is incorporated by reference herein.

### III. Uses of the TNF $\alpha$ Inhibitors of the Invention

The invention provides methods of treating or preventing RSV infection. The invention provides methods for treating or preventing RSV infection in a subject suffering from or at risk of suffering from disorders associated with RSV infection comprising administering a TNF $\alpha$  inhibitor and an additional therapeutic agent. Preferably, the TNF $\alpha$  is human TNF $\alpha$  and the subject is a human subject. In one embodiment, the TNF $\alpha$  inhibitor is D2E7, also referred to as HUMIRA<sup>®</sup> (adalimumab).

As used herein, the term "a disorder in which TNF $\alpha$  activity is detrimental" is intended to include diseases and other disorders in which the presence of TNF $\alpha$  in a subject suffering from the disorder has been shown to be or is suspected of being either responsible for the pathophysiology of the disorder or a factor that contributes to a worsening of the disorder. Accordingly, a disorder in which TNF $\alpha$  activity is detrimental is a disorder in which inhibition of TNF $\alpha$  activity is expected to alleviate the symptoms and/or progression of the disorder. Such disorders may be evidenced, for example, by an increase in the concentration of TNF $\alpha$  in a biological fluid of a subject suffering from the disorder (*e.g.*, an increase in the concentration of TNF $\alpha$  in serum, plasma, synovial fluid, *etc.* of the subject), which can be detected, for example, using an anti-TNF $\alpha$  antibody as described above.

The use of TNF $\alpha$  inhibitors, including antibodies and antibody portions, of the invention in the treatment or prevention of RSV infection or RSV-associated disorders is discussed further below:

TNF $\alpha$  has been implicated as a mediator in RSV-induced illness (see *e.g.*, Rutigliano *et al.* (2004) *J. of Immunol.* 173:3408). The invention provides a method for inhibiting TNF $\alpha$  activity in a subject suffering from an RSV infection, *i.e.*, the invention provides a method for treating RSV infection. The invention also provides a method for treating RSV infection comprising administering a TNF inhibitor and an additional therapeutic agent.

As used herein, the term "RSV infection" refers to a subject who is infected with the RSV virus, and, therefore, may exhibit RSV-associated disorders. As used herein, the term "RSV-associated disorder" refers to any symptom or complication associated with RSV infection. Examples of RSV-associated disorders or symptoms of RSV include, but are not limited to, nasal congestion, nasal flaring, coughing, rapid breathing, breathing difficulty, fever, shortness of breath, wheezing, and hypoxia. Other disorders associated with RSV infection include runny nose and cold-like symptoms. RSV infection may also result in respiratory complications such as pneumonia, bronchitis, and croup.

Subjects at particular risk for RSV infection and the disorders associated with such an infection include young children and infants, the elderly, and those whose immune systems are compromised. Children born prematurely are at high risk for complications associated with RSV infection, particularly those born at less than 28 weeks of gestation.

- 5 Other examples of children at high risk for RSV infection include those with chronic lung disease, such as bronchopulmonary dysplasia, and children with congenital heart disease, such as hemodynamically significant congenital heart disease.

The invention describes use of a TNF inhibitor, *e.g.*, an anti-TNF antibody such as D2E7, in combination with an additional agent for the treatment of RSV infection. A

- 10 TNF inhibitor is used in combination with an additional therapeutic agent known to be effective at preventing and/or treating RSV infection and disorders associated with RSV infection, including neutralizing anti-RSV antibodies such as RespiGam<sup>®</sup> (RSV-IGIV, a human RSV polyclonal antibody), Synagis<sup>®</sup> (palivizumab, RSV monoclonal antibody, see U.S. Patent Nos. 6,656,467 and 5,824,307), and Numax<sup>™</sup> (motavizumab).

- 15 Methods of treatment of RSV infection include acute management and chronic management of the disease. The TNF inhibitor of the invention may be used in combination with at least one additional therapeutic agent known to be effective at acute management of subjects with RSV infection. Such additional agents include adrenaline, bronchodilator drugs (see Cochrane Library Issue 3 (Oxford) 2000), corticosteroids, ribavirin (*NEJM* 325:24-28;1991; *NEJM* 308:1443-1447;1983; *J Pediatrics* 128:422-428; 1996). The TNF inhibitor of the invention may also be used in combination with at least one additional therapeutic agent known to be effective at chronic management of subjects with RSV infection, including, corticosteroids, which may be useful for related asthma-like attacks, ribavirin, which may decrease the incidence of reactive airway disease, and leukotriene antagonists, which may decrease incidence of asthma like symptoms. Additional treatments for subjects having RSV infection include hydration (oral or intravenous), antibiotics, supplemental oxygen, mechanical ventilation, bronchodilators, and epinephrine.

- 20 The methods and compositions of the invention can be used to help prevent serious complications associated with respiratory syncytial virus (RSV) disease. Anti-RSV antibodies, such as palivizumab (Synagis<sup>®</sup>; MedImmune, Inc.), RespiGam<sup>®</sup>, or motavizumab (Numax<sup>™</sup>; MedImmune, Inc.), have been shown to be effective at preventing respiratory disorders caused by RSV in pediatric subjects. Thus, the invention includes a method of preventing disorders associated with RSV infection, comprising administering an anti-RSV antibody, such as palivizumab (Synagis<sup>®</sup>), in combination with an anti-TNF $\alpha$  antibody, including D2E7.

The invention also includes prophylactic treatment comprising methods of preventing RSV infection and disorders associated with RSV infection. As used herein, the term "prevent RSV infection" means a method of preventing disorders associated with RSV infection. RSV infection can be particularly dangerous in certain subjects, including young children and infants, making it beneficial to prevent RSV-associated disorders. Young children and infants, particularly those who are less than a year old and were born prematurely, with other disorders such as heart disease, lung disease, or who are immunocompromised, are at particular risk should they contract RSV. Children at high risk for complications due to RSV infection, such as children with bronchopulmonary dysplasia or hemodynamically significant congenital heart disease, are good candidates for prophylactic treatment methods comprising administration of a neutralizing anti-RSV antibody, such as palivizumab, and an anti-TNF antibody, such as D2E7.

In addition, the methods and compositions of the invention may be used for immunoprophylaxis treatment, which is prevention of RSV infection by immunization. Immunoprophylaxis is a process of providing immunity for individuals who never had RSV infection. Immunoprophylaxis can be accomplished either by administering immunoglobulins or an RSV vaccine. Immunoglobulins are antibodies which are directed against the RSV virus and can provide protection against infection. Immunoprophylactic methods are achieved by administering an anti-RSV antibody to a subject, such as a premature infant, to help increase the subject's immune response to viral infection. Anti-TNF antibodies may be administered in combination with the immunoprophylactic treatment to increase the benefits to the subject at risk of RSV infection.

#### IV. Pharmaceutical Compositions and Pharmaceutical Administration

##### A. *Compositions and Administration*

Antibodies, antibody-portions, and other TNF $\alpha$  inhibitors for use in the treatment and preventive methods of the invention, can be incorporated into pharmaceutical compositions suitable for administration to a subject. Typically, the pharmaceutical composition comprises an antibody, antibody portion, or other TNF $\alpha$  or RSV inhibitor of the invention and a pharmaceutically acceptable carrier. As used herein, "pharmaceutically acceptable carrier" includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like that are physiologically compatible. Examples of pharmaceutically

acceptable carriers include one or more of water, saline, phosphate buffered saline, dextrose, glycerol, ethanol and the like, as well as combinations thereof. In many cases, it is preferable to include isotonic agents, for example, sugars, polyalcohols such as mannitol, sorbitol, or sodium chloride in the composition. Pharmaceutically acceptable carriers may further comprise minor amounts of auxiliary substances such as wetting or emulsifying agents, preservatives or buffers, which enhance the shelf life or effectiveness of the antibody, antibody portion, or other TNF $\alpha$  inhibitor.

The compositions for use in the methods of the invention may be in a variety of forms. These include, for example, liquid, semi-solid and solid dosage forms, such as liquid solutions (*e.g.*, injectable and infusible solutions), dispersions or suspensions, tablets, pills, powders, liposomes and suppositories. The preferred form depends on the intended mode of administration and therapeutic application. Typical preferred compositions are in the form of injectable or infusible solutions, such as compositions similar to those used for passive immunization of humans with other antibodies or other TNF $\alpha$  inhibitors. The preferred mode of administration is parenteral (*e.g.*, intravenous, subcutaneous, intraperitoneal, intramuscular). In a preferred embodiment, the antibody or other TNF $\alpha$  inhibitor is administered by intravenous infusion or injection. In another preferred embodiment, the antibody or other TNF $\alpha$  inhibitor is administered by intramuscular or subcutaneous injection.

Therapeutic compositions typically must be sterile and stable under the conditions of manufacture and storage. The composition can be formulated as a solution, microemulsion, dispersion, liposome, or other ordered structure suitable to high drug concentration. Sterile injectable solutions can be prepared by incorporating the active compound (*i.e.*, antibody, antibody portion, or other TNF $\alpha$  inhibitor) in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle that contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and freeze-drying that yields a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof. The proper fluidity of a solution can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prolonged absorption of injectable compositions can be brought about by including in the composition an agent that delays absorption, for example, monostearate salts and gelatin.

Supplementary active compounds can also be incorporated into the compositions. In certain embodiments, an antibody or antibody portion for use in the methods of the invention is coformulated with and/or coadministered with one or more additional therapeutic agents, including an RSV inhibitor or antagonist. For example, an anti-hTNF $\alpha$  antibody or antibody portion of the invention may be coformulated and/or coadministered with one or more anti-RSV antibodies or one or more additional antibodies that bind other targets (*e.g.*, antibodies that bind other cytokines or that bind cell surface molecules), one or more cytokines, soluble TNF $\alpha$  receptor (see *e.g.*, PCT Publication No. WO 94/06476) and/or one or more chemical agents that inhibit hTNF $\alpha$  production or activity (such as cyclohexane-ylidene derivatives as described in PCT Publication No. WO 93/19751) or any combination thereof. Furthermore, one or more antibodies of the invention may be used in combination with two or more of the foregoing therapeutic agents. Such combination therapies may advantageously utilize lower dosages of the administered therapeutic agents, thus avoiding possible side effects, complications or low level of response by the patient associated with the various monotherapies.

In one embodiment, the invention includes pharmaceutical compositions comprising an effective amount of a TNF $\alpha$  inhibitor and a pharmaceutically acceptable carrier, wherein the effective amount of the TNF $\alpha$  inhibitor may be effective to treat a TNF $\alpha$ -related disorder, including, for example, RSV infection. In one embodiment, the antibody or antibody portion for use in the methods of the invention is incorporated into a pharmaceutical formulation as described in PCT/IB03/04502 and U.S. Appln. No. 10/222140, incorporated by reference herein. This formulation includes a concentration 50 mg/ml of the antibody D2E7, wherein one pre-filled syringe contains 40 mg of antibody for subcutaneous injection. In another embodiment, the formulation of the invention includes D2E7 and an anti-RSV antibody. In an additional embodiment, the formulation of the invention includes D2E7 and palivizumab (Synagis<sup>®</sup>), RSV-IGIV (Respigam<sup>®</sup>), or motavizumab (Numax<sup>™</sup>).

The antibody D2E7 may also be administered in combination with an anti-RSV antibody, such as palivizumab, for the prevention of RSV-associated disorders. In one embodiment of the invention, D2E7 and palivizumab are co-administered for prevention or treatment of RSV infection. In another embodiment, D2E7 and palivizumab are co-formulated for prevention or treatment of RSV infection.

The antibodies, antibody-portions, and other TNF $\alpha$  inhibitors of the present invention can be administered by a variety of methods known in the art, although for many therapeutic applications, the preferred route/mode of administration is subcutaneous injection. In another embodiment, administration is via intravenous injection or infusion.



As will be appreciated by the skilled artisan, the route and/or mode of administration will vary depending upon the desired results. In certain embodiments, the active compound may be prepared with a carrier that will protect the compound against rapid release, such as a controlled release formulation, including implants, transdermal patches, and  
5 microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Many methods for the preparation of such formulations are patented or generally known to those skilled in the art. See, *e.g.*, *Sustained and Controlled Release Drug Delivery Systems*, J.R. Robinson, ed., Marcel  
10 Dekker, Inc., New York, 1978.

The TNF $\alpha$  antibodies of the invention can also be administered in the form of protein crystal formulations which include a combination of protein crystals encapsulated within a polymeric carrier to form coated particles. The coated particles of the protein crystal formulation may have a spherical morphology and be microspheres of up to 500  
15 micro meters in diameter or they may have some other morphology and be microparticulates. The enhanced concentration of protein crystals allows the antibody of the invention to be delivered subcutaneously. In one embodiment, the TNF $\alpha$  antibodies of the invention are delivered via a protein delivery system, wherein one or more of a protein crystal formulation or composition, is administered to a subject with a TNF $\alpha$ -related  
20 disorder. Compositions and methods of preparing stabilized formulations of whole antibody crystals or antibody fragment crystals are also described in WO 02/072636, which is incorporated by reference herein. In one embodiment, a formulation comprising the crystallized antibody fragments described in PCT/IB03/04502 and U.S. Appln. No. 10/222140, incorporated by reference herein, is used to treat a RSV infection using the  
25 multiple-variable dose methods of the invention..

In certain embodiments, an antibody, antibody portion, or other TNF $\alpha$  inhibitor of the invention may be orally administered, for example, with an inert diluent or an assimilable edible carrier. The compound (and other ingredients, if desired) may also be enclosed in a hard or soft shell gelatin capsule, compressed into tablets, or incorporated  
30 directly into the subject's diet. For oral therapeutic administration, the compounds may be incorporated with excipients and used in the form of ingestible tablets, buccal tablets, troches, capsules, elixirs, suspensions, syrups, wafers, and the like. To administer a compound of the invention by other than parenteral administration, it may be necessary to coat the compound with, or co-administer the compound with, a material to prevent its  
35 inactivation.

The pharmaceutical compositions of the invention may include a "therapeutically effective amount" or a "prophylactically effective amount" of an antibody or antibody portion of the invention. A "therapeutically effective amount" refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired therapeutic result. A therapeutically effective amount of the antibody, antibody portion, or other TNF $\alpha$  inhibitor may vary according to factors such as the disease state, age, sex, and weight of the individual, and the ability of the antibody, antibody portion, other TNF $\alpha$  inhibitor to elicit a desired response in the individual. A therapeutically effective amount is also one in which any toxic or detrimental effects of the antibody, antibody portion, or other TNF $\alpha$  inhibitor are outweighed by the therapeutically beneficial effects. A "prophylactically effective amount" refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired prophylactic result. Typically, since a prophylactic dose is used in subjects prior to or at an earlier stage of disease, the prophylactically effective amount will be less than the therapeutically effective amount.

Dosage regimens may be adjusted to provide the optimum desired response (*e.g.*, a therapeutic or prophylactic response). For example, a single bolus may be administered, several divided doses may be administered over time or the dose may be proportionally reduced or increased as indicated by the exigencies of the therapeutic situation. It is especially advantageous to formulate parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the mammalian subjects to be treated; each unit containing a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the dosage unit forms of the invention are dictated by and directly dependent on (a) the unique characteristics of the active compound and the particular therapeutic or prophylactic effect to be achieved, and (b) the limitations inherent in the art of compounding such an active compound for the treatment of sensitivity in individuals.

An exemplary, non-limiting range for a therapeutically or prophylactically effective amount of an antibody or antibody portion of the invention is 10-180 mg, more preferably 20-160 mg and most preferably about 80 mg. In one embodiment, the therapeutically effective amount of an antibody or portion thereof for use in the methods of the invention is 40 mg. In another embodiment, the therapeutically effective amount of an antibody or portion thereof for use in the methods of the invention is 80 mg. In still another embodiment, the therapeutically effective amount of an antibody or portion thereof for use in the methods of the invention is 160 mg. Ranges intermediate to the

above recited dosages, *e.g.* about 78.5 – 81.5, are also intended to be part of this invention. For example, ranges of values using a combination of any of the above recited values as upper and/or lower limits are intended to be included.

In one embodiment, the invention provides a single dose method for treating  
5 RSV infection, comprising administering to a subject in need thereof a single dose of a TNF $\alpha$  inhibitor, such as a human antibody. In one embodiment, the TNF $\alpha$  inhibitor is the anti-TNF $\alpha$  antibody D2E7. The single dose of TNF $\alpha$  inhibitor can be any therapeutically or prophylactically effective amount. In one embodiment, a subject is administered either a 20 mg, a 40 mg, or an 80 mg single dose of D2E7. The single dose  
10 may be administered through any route, including, for example, subcutaneous administration. Multiple variable dose methods of treatment or prevention can also be used, and are described in PCT/US2005/012007, incorporated by reference herein. Low dose methods through which the anti-TNF antibody may be administered for the treatment of RSV infection are described in PCT publication no. WO 04/037205.

15 It is to be noted that dosage values may vary with the type and severity of the condition to be alleviated. It is to be further understood that for any particular subject, specific dosage regimens should be adjusted over time according to the individual need and the professional judgment of the person administering or supervising the administration of the compositions, and that dosage ranges set forth herein are  
20 exemplary only and are not intended to limit the scope or practice of the claimed composition.

The invention also pertains to packaged pharmaceutical compositions or kits for administering the anti-TNF and anti-RSV antibodies of the invention. In one embodiment of the invention, the kit comprises a TNF $\alpha$  inhibitor, such as an antibody, an second  
25 pharmaceutical composition comprising an additional therapeutic agent, and instructions for administration for treatment of RSV infection or prevention of RSV-associated disorders. The instructions may describe how, *e.g.*, subcutaneously, and when, *e.g.*, at week 0 and week 2, the different doses of TNF $\alpha$  inhibitor and/or the additional therapeutic agent shall be administered to a subject for treatment.

30 Another aspect of the invention pertains to kits containing a pharmaceutical composition comprising an anti-TNF $\alpha$  antibody and a pharmaceutically acceptable carrier and one or more pharmaceutical compositions each comprising a drug useful for treating RSV infection and a pharmaceutically acceptable carrier. Alternatively, the kit comprises a single pharmaceutical composition comprising an anti-TNF $\alpha$  antibody, one  
35 or more drugs useful for treating RSV infection or prevention of RSV-associated disorders and a pharmaceutically acceptable carrier. The kits contain instructions for

dosing of the pharmaceutical compositions for the treatment of RSV infection or prevention of RSV-associated disorders in which the administration of an anti-TNF $\alpha$  antibody is beneficial.

5 The package or kit alternatively can contain the TNF $\alpha$  inhibitor and it can be promoted for use, either within the package or through accompanying information, for the uses or treatment of the disorders described herein. The packaged pharmaceuticals or kits further can include a second agent (as described herein) packaged with or copromoted with instructions for using the second agent with a first agent (as described herein).

10

**B. *Additional therapeutic agents***

The invention pertains to pharmaceutical compositions and methods of use thereof for the treatment or prevention of RSV infection or RSV-associated disorders. The pharmaceutical compositions comprise a first agent that prevents or treats RSV  
15 infection. The pharmaceutical composition also may comprise a second agent that is an active pharmaceutical ingredient; that is, the second agent is therapeutic and its function is beyond that of an inactive ingredient, such as a pharmaceutical carrier, preservative, diluent, or buffer. The second agent may be useful in treating or preventing TNF $\alpha$ -related disorders. The second agent may diminish or treat at least one symptom(s)  
20 associated with the targeted disease. The first and second agents may exert their biological effects by similar or unrelated mechanisms of action; or either one or both of the first and second agents may exert their biological effects by a multiplicity of mechanisms of action. A pharmaceutical composition may also comprise a third compound, or even more yet, wherein the third (and fourth, etc.) compound has the same  
25 characteristics of a second agent.

It should be understood that the pharmaceutical compositions described herein may have the first and second, third, or additional agents in the same pharmaceutically acceptable carrier or in a different pharmaceutically acceptable carrier for each described embodiment. It further should be understood that the first, second, third and additional  
30 agent may be administered simultaneously or sequentially within described embodiments. Alternatively, a first and second agent may be administered simultaneously, and a third or additional agent may be administered before or after the first two agents.

The combination of agents used within the methods and pharmaceutical  
35 compositions described herein may have a therapeutic additive or synergistic effect on the condition(s) or disease(s) targeted for treatment. The combination of agents used

within the methods or pharmaceutical compositions described herein also may reduce a detrimental effect associated with at least one of the agents when administered alone or without the other agent(s) of the particular pharmaceutical composition. For example, the toxicity of side effects of one agent may be attenuated by another agent of the composition, thus allowing a higher dosage, improving patient compliance, and improving therapeutic outcome. The additive or synergistic effects, benefits, and advantages of the compositions apply to classes of therapeutic agents, either structural or functional classes, or to individual compounds themselves.

Supplementary active compounds can also be incorporated into the compositions.

10 In certain embodiments, an antibody or antibody portion of the invention is coformulated with and/or coadministered with one or more additional therapeutic agents that are useful for treating or preventing RSV infection. For example, an anti-hTNF $\alpha$  antibody, antibody portion, or other TNF $\alpha$  inhibitor of the invention may be coformulated and/or coadministered with one or more additional antibodies that bind other targets (*e.g.*, antibodies that bind other cytokines or that bind cell surface molecules), one or more cytokines, soluble TNF $\alpha$  receptor (see *e.g.*, PCT Publication No. WO 94/06476) and/or one or more chemical agents that inhibit hTNF $\alpha$  production or activity (such as cyclohexane-ylidene derivatives as described in PCT Publication No. WO 93/19751). Furthermore, one or more antibodies or other TNF $\alpha$  inhibitors of the invention may be used in combination with two or more of the foregoing therapeutic agents. Such combination therapies may advantageously utilize lower dosages of the administered therapeutic agents, thus avoiding possible toxicities or complications associated with the various monotherapies.

The TNF $\alpha$  inhibitors of the invention may be used in combination with additional therapeutic agents for the treatment or prevention of RSV infection.

25 Additional agents used to treat RSV infection include, but are not limited to, adrenaline, bronchodilator drugs, corticosteroids, ribavirin, leukotriene antagonists, Respigam (an RSV polyclonal antibody), Synagis (RSV monoclonal antibody), and Numax. In addition, Respigam<sup>®</sup> (a human RSV antibody), Synagis<sup>®</sup> (RSV monoclonal antibody), and Numax<sup>™</sup> may also be used prophylactically for RSV infection.

Other nonlimiting examples of therapeutic agents with which an antibody, antibody portion, or other TNF $\alpha$  inhibitor of the invention can be combined include the following: non-steroidal anti-inflammatory drug(s) (NSAIDs); cytokine suppressive anti-inflammatory drug(s) (CSAIDs); CDP-571/BAY-10-3356 (humanized anti-TNF $\alpha$  antibody; Celltech/Bayer); cA2/infliximab (chimeric anti-TNF $\alpha$  antibody; Centocor); 75 kDTNFR-IgG/etanercept (75 kD TNF receptor-IgG fusion protein; Immunex; see *e.g.*,

35

*Arthritis & Rheumatism* (1994) Vol. 37, S295; *J. Invest. Med.* (1996) Vol. 44, 235A); 55  
 kdTNF-IgG (55 kD TNF receptor-IgG fusion protein; Hoffmann-LaRoche); IDEC-  
 CE9.1/SB 210396 (non-depleting primatized anti-CD4 antibody; IDEC/SmithKline; see  
*e.g.*, *Arthritis & Rheumatism* (1995) Vol. 38, S185); DAB 486-IL-2 and/or DAB 389-IL-  
 5 2 (IL-2 fusion proteins; Seragen; see *e.g.*, *Arthritis & Rheumatism* (1993) Vol. 36, 1223);  
 Anti-Tac (humanized anti-IL-2R $\alpha$ ; Protein Design Labs/Roche); IL-4 (anti-inflammatory  
 cytokine; DNAX/Schering); IL-10 (SCH 52000; recombinant IL-10, anti-inflammatory  
 cytokine; DNAX/Schering); IL-4; IL-10 and/or IL-4 agonists (*e.g.*, agonist antibodies);  
 IL-1RA (IL-1 receptor antagonist; Synergen/Amgen); TNF-bp/s-TNF (soluble TNF  
 10 binding protein; see *e.g.*, *Arthritis & Rheumatism* (1996) Vol. 39, No. 9 (supplement),  
 S284; *Amer. J. Physiol. - Heart and Circulatory Physiology* (1995) Vol. 268, pp. 37-42);  
 R973401 (phosphodiesterase Type IV inhibitor; see *e.g.*, *Arthritis & Rheumatism* (1996)  
 Vol. 39, No. 9 (supplement), S282); MK-966 (COX-2 Inhibitor; see *e.g.*, *Arthritis &*  
*Rheumatism* (1996) Vol. 39, No. 9 (supplement), S81); Iloprost (see *e.g.*, *Arthritis &*  
 15 *Rheumatism* (1996) Vol. 39, No. 9 (supplement), S82); methotrexate; thalidomide (see  
*e.g.*, *Arthritis & Rheumatism* (1996) Vol. 39, No. 9 (supplement), S282) and  
 thalidomide-related drugs (*e.g.*, Celgen); leflunomide (anti-inflammatory and cytokine  
 inhibitor; see *e.g.*, *Arthritis & Rheumatism* (1996) Vol. 39, No. 9 (supplement), S131;  
*Inflammation Research* (1996) Vol. 45, pp. 103-107); tranexamic acid (inhibitor of  
 20 plasminogen activation; see *e.g.*, *Arthritis & Rheumatism* (1996) Vol. 39, No. 9  
 (supplement), S284); T-614 (cytokine inhibitor; see *e.g.*, *Arthritis & Rheumatism* (1996)  
 Vol. 39, No. 9 (supplement), S282); prostaglandin E1 (see *e.g.*, *Arthritis & Rheumatism*  
 (1996) Vol. 39, No. 9 (supplement), S282); Tenidap (non-steroidal anti-inflammatory  
 drug; see *e.g.*, *Arthritis & Rheumatism* (1996) Vol. 39, No. 9 (supplement), S280);  
 25 Naproxen (non-steroidal anti-inflammatory drug; see *e.g.*, *Neuro Report* (1996) Vol. 7,  
 pp. 1209-1213); Meloxicam (non-steroidal anti-inflammatory drug); Ibuprofen (non-  
 steroidal anti-inflammatory drug); Piroxicam (non-steroidal anti-inflammatory drug);  
 Diclofenac (non-steroidal anti-inflammatory drug); Indomethacin (non-steroidal anti-  
 inflammatory drug); Sulfasalazine (see *e.g.*, *Arthritis & Rheumatism* (1996) Vol. 39, No.  
 30 9 (supplement), S281); Azathioprine (see *e.g.*, *Arthritis & Rheumatism* (1996) Vol. 39,  
 No. 9 (supplement), S281); ICE inhibitor (inhibitor of the enzyme interleukin-1 $\beta$   
 converting enzyme); zap-70 and/or lck inhibitor (inhibitor of the tyrosine kinase zap-70  
 or lck); VEGF inhibitor and/or VEGF-R inhibitor (inhibitors of vascular endothelial cell  
 growth factor or vascular endothelial cell growth factor receptor; inhibitors of  
 35 angiogenesis); corticosteroid anti-inflammatory drugs (*e.g.*, SB203580); TNF-convertase  
 inhibitors; anti-IL-12 antibodies; anti-IL-18 antibodies; interleukin-11 (see *e.g.*, *Arthritis*

- & *Rheumatism* (1996) Vol. 39, No. 9 (supplement), S296); interleukin-13 (see *e.g.*, *Arthritis & Rheumatism* (1996) Vol. 39, No. 9 (supplement), S308); interleukin-17 inhibitors (see *e.g.*, *Arthritis & Rheumatism* (1996) Vol. 39, No. 9 (supplement), S120); gold; penicillamine; chloroquine; hydroxychloroquine; chlorambucil;
- 5 cyclophosphamide; cyclosporine; total lymphoid irradiation; anti-thymocyte globulin; anti-CD4 antibodies; CD5-toxins; orally-administered peptides and collagen; lobenzarit disodium; Cytokine Regulating Agents (CRAs) HP228 and HP466 (Houghten Pharmaceuticals, Inc.); ICAM-1 antisense phosphorothioate oligodeoxynucleotides (ISIS 2302; Isis Pharmaceuticals, Inc.); soluble complement receptor 1 (TP10; T Cell
- 10 Sciences, Inc.); prednisone; orgotein; glycosaminoglycan polysulphate; minocycline; anti-IL2R antibodies; marine and botanical lipids (fish and plant seed fatty acids; see *e.g.*, DeLuca *et al.* (1995) *Rheum. Dis. Clin. North Am.* 21:759-777); auranofin; phenylbutazone; meclofenamic acid; flufenamic acid; intravenous immune globulin; zileuton; mycophenolic acid (RS-61443); tacrolimus (FK-506); sirolimus (rapamycin);
- 15 amiprilose (therafectin); cladribine (2-chlorodeoxyadenosine); azaribine; methotrexate; antivirals; and immune modulating agents. Any of the above-mentioned agents can be administered in combination with the TNF $\alpha$  antibody of the invention to treat or prevent RSV infection.

- In yet another embodiment, the TNF $\alpha$  antibody of the invention is administered
- 20 in combination with an antibiotic or antiinfective agent to treat or prevent RSV infection. Antiinfective agents include those agents known in the art to treat viral, fungal, parasitic or bacterial infections. The term, "antibiotic," as used herein, refers to a chemical substance that inhibits the growth of, or kills, microorganisms. Encompassed by this term are antibiotic produced by a microorganism, as well as synthetic antibiotics (*e.g.*,
- 25 analogs) known in the art. Antibiotics include, but are not limited to, clarithromycin (Biaxin<sup>®</sup>), ciprofloxacin (Cipro<sup>®</sup>), and metronidazole (Flagyl<sup>®</sup>). The TNF $\alpha$  antibody of the invention may also be administered in combination with an agent for the treatment or prevention of a viral disorder, including RSV infection. For example, the TNF $\alpha$  antibody of the invention may be administered in combination with palivizumab
- 30 (Synagis<sup>®</sup>) for the prevention of RSV disorders.

- Any one of the above-mentioned therapeutic agents, alone or in combination therewith, can be administered to a subject suffering from a RSV infection, in combination with the TNF $\alpha$  antibody of the invention. In addition, any one of the above-mentioned therapeutic agents, alone or in combination, can be administered to a
- 35 subject at risk for developing RSV infection, in combination with an anti-TNF antibody.

## EXAMPLES

### **Example 1: Criteria for choosing patients for prophylactic treatment of RSV**

Pediatric patients may be administered a combination treatment comprising a  
5 TNF inhibitor, such as an anti-TNF $\alpha$  antibody, *i.e.*, D2E7, and an additional agent, such  
as Synagis<sup>®</sup> or Numax<sup>™</sup>, for the prevention of RSV infection and disorders associated  
with RSV infection. Generally, children in need of prophylactic treatment are identified  
according to either their physical symptoms, their age and prematurity history, or both.  
Children are assessed according to their risk for RSV infection and complications  
10 associated from such infection, and are chosen for prophylactic treatment according to  
the following criteria:

#### *History of premature birth*

Children in need of prophylactic treatment for RSV infection include infants born  
15 prematurely, as severe complications from RSV infection are more likely to develop in  
prematurely born infants. Premature infants at the highest risk are those born  
prematurely at <28 weeks of gestation. Infants born between 28-32 weeks of gestation  
are at moderate risk for RSV infection and symptoms associated with such infection,  
while those infants born between 23 and 35 weeks are at lesser risk of contracting a  
20 severe RSV infection. In addition to being born prematurely, candidate patients are less  
than one year old.

#### *Lung disease*

Another criterion for choosing patients for prophylactic treatment of RSV  
25 infection includes chronic lung disease (CLD), more specifically bronchopulmonary  
dysplasia or BPD. Bronchopulmonary dysplasia involves abnormal development of lung  
tissue, and is a disease in infants characterized by inflammation and scarring in the  
lungs. BPD develops most often in premature babies, who are born with  
underdeveloped lungs.

30

#### *Heart disease*

In addition to premature birth status, age, and lung disease, congenital heart  
disease is also indicative that a patient may benefit from preventative treatment of RSV  
comprising administration of a TNF inhibitor, such as an anti-TNF $\alpha$  antibody, and an  
35 additional agent, such as Synagis<sup>®</sup> or Numax<sup>™</sup>. Specifically, infants diagnosed with



hemodynamically significant congenital heart disease in the first 2 years of life are candidates for preventative RSV treatment.

5 It should be noted that premature infants who are less than a year old, as described above, who have also developed BPD or were born with a hemodynamically significant congenital heart disease should be considered as candidates for the prophylactic treatment of the invention. Infants who exhibit BPD or have a hemodynamically significant congenital heart disease but were not born prematurely should also be considered for preventative treatment of RSV.

10

### **Example 2: Treatment of RSV infection**

In cases where patients are treated for RSV infection, wherein the patient exhibits RSV-associated disorders, the following standard of care is used. The subject is administered an anti-TNF antibody and an additional therapeutic agent. The additional  
15 therapeutic agent may include, but is not limited to, an antibiotic, hydration, supplemental oxygen, a bronchodilator (including albuterol, salbutamol), epinephrine, a corticosteroid, a leukotriene inhibitor, RespiGam<sup>®</sup>, Synagis<sup>®</sup>, or Numax<sup>™</sup>. The treatment is further supported by the following activities:

### **20 *Standard Therapy for RSV***

Present treatment for RSV infection is supportive, and includes oral hydration and feeding and close monitoring by a medical professional. Hydration is oral or intravenous, if necessary. The subject is monitored with respect to oxygenation, circulatory status, and metabolic balance. The medical professional also maintains  
25 surveillance for superimposed bacterial infection, and antibiotics are administered if needed. In addition, supplemental oxygen and/or if needed mechanical ventilation is administered if needed.

Bronchodilators (albuterol, salbutamol) may also be used, both by inhaled and/or parenteral route. In a small percentage (1%) of RSV infected subjects, hospitalization  
30 will be required for RSV bronchiolitis. In these cases, supplemental oxygen may be needed and monitoring of the respiratory status is required. Additional bronchodilators may be added to treat the reactive airway component of the disease.

Additional agents which may be administered to the RSV-infected subject include epinephrine, corticosteroids, and leukotriene inhibitors.

35

**EQUIVALENTS**

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following  
5 claims. The contents of all references, patents and published patent applications cited throughout this application are incorporated herein by reference

What is claimed:

1. A method for treating a human subject suffering from respiratory syncytial virus (RSV) infection, comprising administering to the subject an anti-TNF $\alpha$  antibody and an additional therapeutic agent, such that the RSV infection is treated.
2. The method of claim 1, wherein the anti-TNF $\alpha$  antibody is a human antibody.
3. A method for treating a human subject suffering from RSV infection, comprising administering to the subject an anti-TNF $\alpha$  antibody and an additional therapeutic agent, such that the RSV infection is treated, wherein the antibody is an isolated human antibody, or an antigen-binding portion thereof, that dissociates from human TNF $\alpha$  with a  $K_d$  of  $1 \times 10^{-8}$  M or less and a  $K_{off}$  rate constant of  $1 \times 10^{-3} \text{ s}^{-1}$  or less, both determined by surface plasmon resonance, and neutralizes human TNF $\alpha$  cytotoxicity in a standard *in vitro* L929 assay with an  $IC_{50}$  of  $1 \times 10^{-7}$  M or less.
4. A method for treating a human subject suffering from RSV infection, comprising administering to the subject an anti-TNF $\alpha$  antibody and an additional therapeutic agent, such that the RSV infection is treated, wherein the antibody is an isolated human antibody, or antigen-binding portion thereof, with the following characteristics:
  - a) dissociates from human TNF $\alpha$  with a  $K_{off}$  rate constant of  $1 \times 10^{-3} \text{ s}^{-1}$  or less, as determined by surface plasmon resonance;
  - b) has a light chain CDR3 domain comprising the amino acid sequence of SEQ ID NO: 3, or modified from SEQ ID NO: 3 by a single alanine substitution at position 1, 4, 5, 7 or 8 or by one to five conservative amino acid substitutions at positions 1, 3, 4, 6, 7, 8 and/or 9;
  - c) has a heavy chain CDR3 domain comprising the amino acid sequence of SEQ ID NO: 4, or modified from SEQ ID NO: 4 by a single alanine substitution at position 2, 3, 4, 5, 6, 8, 9, 10 or 11 or by one to five conservative amino acid substitutions at positions 2, 3, 4, 5, 6, 8, 9, 10, 11 and/or 12.

5. A method for treating a human subject suffering from RSV infection, comprising administering to the subject an anti-TNF $\alpha$  antibody and an additional therapeutic agent, such that the RSV infection is treated,, wherein the antibody is an isolated human antibody, or an antigen binding portion thereof, with a light chain variable region (LCVR) comprising the amino acid sequence of SEQ ID NO: 1 and a heavy chain variable region (HCVR) comprising the amino acid sequence of SEQ ID NO: 2
6. A method for treating a human subject suffering from RSV infection, comprising administering to the subject an anti-TNF $\alpha$  antibody and an additional therapeutic agent, wherein the antibody is D2E7.
7. The method of any one of claims 1-6, wherein the additional therapeutic agent is selected from the group consisting of adrenaline, a bronchodilator drug, a corticosteroid, ribavirin, and a leukotriene antagonist.
8. A method for preventing an RSV-associated disorder in a human subject, comprising administering to the subject an anti-TNF $\alpha$  antibody and an additional therapeutic agent.
9. The method of claim 8, wherein the anti-TNF $\alpha$  antibody is a human antibody.
10. A method for preventing an RSV-associated disorder in a human subject, comprising administering to the subject an anti-TNF $\alpha$  antibody and an additional therapeutic agent, wherein the antibody is an isolated human antibody, or an antigen-binding portion thereof, that dissociates from human TNF $\alpha$  with a  $K_d$  of  $1 \times 10^{-8}$  M or less and a  $K_{off}$  rate constant of  $1 \times 10^{-3} \text{ s}^{-1}$  or less, both determined by surface plasmon resonance, and neutralizes human TNF $\alpha$  cytotoxicity in a standard *in vitro* L929 assay with an  $IC_{50}$  of  $1 \times 10^{-7}$  M or less.
11. A method for preventing an RSV-associated disorder in a human subject, comprising administering to the subject an anti-TNF $\alpha$  antibody and an additional

therapeutic agent, wherein the antibody is an isolated human antibody, or antigen-binding portion thereof, with the following characteristics:

a) dissociates from human TNF $\alpha$  with a  $K_{off}$  rate constant of  $1 \times 10^{-3} \text{ s}^{-1}$  or less, as determined by surface plasmon resonance;

5        b) has a light chain CDR3 domain comprising the amino acid sequence of SEQ ID NO: 3, or modified from SEQ ID NO: 3 by a single alanine substitution at position 1, 4, 5, 7 or 8 or by one to five conservative amino acid substitutions at positions 1, 3, 4, 6, 7, 8 and/or 9;

10       c) has a heavy chain CDR3 domain comprising the amino acid sequence of SEQ ID NO: 4, or modified from SEQ ID NO: 4 by a single alanine substitution at position 2, 3, 4, 5, 6, 8, 9, 10 or 11 or by one to five conservative amino acid substitutions at positions 2, 3, 4, 5, 6, 8, 9, 10, 11 and/or 12.

12.    A method for preventing an RSV-associated disorder in a human subject,  
15    comprising administering to the subject an anti-TNF $\alpha$  antibody and an additional therapeutic agent, wherein the antibody is an isolated human antibody, or an antigen binding portion thereof, with a light chain variable region (LCVR) comprising the amino acid sequence of SEQ ID NO: 1 and a heavy chain variable region (HCVR) comprising the amino acid sequence of SEQ ID NO: 2

20

13.    A method for preventing an RSV-associated disorder in a human subject, comprising administering to the subject an anti-TNF $\alpha$  antibody and an additional therapeutic agent, wherein the antibody is D2E7.

25    14.    The method of any one of claims 10-13, where the additional therapeutic agent is an anti-RSV antibody.

15.    The method of claim 14, wherein the anti-RSV antibody is palivizumab (Synagis<sup>®</sup>).

30

16.    The method of claim 14, wherein the anti-RSV antibody is Respigam or Numax.

17. A method for treating RSV infection or preventing RSV-associated disorders in a human subject, comprising administering to the subject a combination treatment comprising a D2E7 antibody and a palivizumab antibody (Synagis).
- 5 18. The method of claim 17, wherein the D2E7 antibody and the palivizumab antibody are co-formulated.
19. The method of any one of claims 1-18, wherein the subject is a child or an infant.
- 10 20. A pharmaceutical composition comprising D2E7, palivizumab, and a pharmaceutically acceptable carrier.
21. A kit comprising:
- 15 a) a pharmaceutical composition comprising an anti-TNF $\alpha$  antibody and a pharmaceutically acceptable carrier;
- b) at least one pharmaceutical composition each comprising an additional therapeutic agent and a pharmaceutically acceptable carrier; and
- c) instructions for administration of the pharmaceutical composition of (a) and (b) for the treatment of RSV infection or prevention of RSV-associated disorders.
- 20 22. The kit of claim 21, wherein the anti-TNF $\alpha$  antibody is D2E7.
23. A kit comprising:
- 25 a) a pharmaceutical composition comprising D2E7 and a pharmaceutically acceptable carrier;
- b) a pharmaceutical composition comprising an anti-RSV antibody and a pharmaceutically acceptable carrier; and
- c) instructions for administration of D2E7 and the anti-RSV antibody for the prevention of RSV-associated disorders.
- 30 24. The kit of claim 23, wherein the anti-RSV antibody is palivizumab (Synagis).

25. The kit of claim 23, wherein the anti-RSV antibody is Respigam or Numax.

26. A formulation comprising D2E7 and palivizumab for the treatment of RSV  
5 infection or prevention of RSV-associated disorders.

27. The formulation of claim 26 which is in liquid form.

10

15

## BBI-219CPPC SEQLIST

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Pollack, Paul

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&lt;213&gt; Artificial Sequence

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&lt;213&gt; Artificial Sequence

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&lt;212&gt; PRT

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&lt;211&gt; 12

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

&lt;220&gt;

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&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

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&lt;223&gt; 3C-H2 heavy chain variable region CDR3

&lt;400&gt; 34

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&lt;211&gt; 12

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&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; VH1-D2.N heavy chain variable region CDR3

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(22) International Filing Date: 6 October 2005 (06.10.2005)

(25) Filing Language: English

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(71) Applicant (for all designated States except US): **ABBOTT BIOTECHNOLOGY LTD.** [GB/GB]; Clarendon House, 2 Church Street, HM 11 Hamilton (BM).

(72) Inventors; and

(75) Inventors/Applicants (for US only): **HOFFMAN, Rebecca, S.** [US/US]; 1300 Elmwood Avenue, Wilmette, IL 60091 (US). **CHARTASH, Elliot, K.** [US/US]; 28 Edinborough Drive, Randolph, NJ 07078 (US). **POLLACK, Paul, F.** [US/US]; 23 Ronald Terrace, West Orange, NJ 07052 (US).

(74) Agents: **DECONTI, Giulio, A.** et al.; Lahive & Cockfield, LLP, One Post Office Square, Boston, MA 02109-2127 (US).

(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN,

CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, LY, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

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**Previous Correction:**

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For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: TREATMENT OF RESPIRATORY SYNCYTIAL VIRUS (RSV) INFECTION

(57) Abstract: The invention describes methods of treating and preventing respiratory syncytial virus (RSV) infection comprising administering a TNF inhibitor and an additional therapeutic agent. The invention also describes methods of treating and preventing respiratory syncytial virus (RSV) infection comprising administering an anti-TNF antibody and an anti-RSV antibody.



WO 2006/041970 A3

## INTERNATIONAL SEARCH REPORT

International application No.

PCT/US05/35910

**A. CLASSIFICATION OF SUBJECT MATTER**

IPC: A61K 39/395( 2006.01),39/00( 2006.01)

USPC: 424/158.1,178.1;530/388.23

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/158.1,178.1;530/388.23

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)  
Please See Continuation Sheet**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US RE37,525 (PRINCE et al.) 22 January 2002 (22.01.2002), see column 5, lines 13-18, and 38-41, column 6, lines 14-16.	1-18 and 20-27
Y	LORENZ, H-M., Perspectives for TNF- $\alpha$ -targeting therapies. Arthritis Research. 2002, Vol. 4, (Suppl 3), pages S17-S24, see entire document.	1-18 and 20-27
Y	JOHNSON, S., et al., A direct comparison of the activities of two humanized respiratory syncytial virus monoclonal antibodies: MEDI-493 and RSHZ19. The Journal of Infectious Diseases. 1999, Vol. 180, Iss. 1, pages 35-41, see entire document.	1-18 and 20-27
A	TAYLOR, P.C., Anti-TNF $\alpha$ Therapy for Rheumatoid Arthritis: an Update. Internal Medicine. 2003, Vol. 42, No. 1, pages 15-20.	1-18 and 20-27

☐ Further documents are listed in the continuation of Box C.

See patent family annex.

**\* Special categories of cited documents:**

"A"	document defining the general state of the art which is not considered to be of particular relevance	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"E"	earlier application or patent published on or after the international filing date	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"O"	document referring to an oral disclosure, use, exhibition or other means	"&"	document member of the same patent family
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search

22 March 2007 (22.03.2007)

Date of mailing of the international search report

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Name and mailing address of the ISA/US

Mail Stop PCT, Attn: ISA/US  
Commissioner for Patents  
P.O. Box 1450  
Alexandria, Virginia 22313-1450

Facsimile No. (571) 273-3201

Authorized officer

Myron G. Hill  
Telephone No. 703-308-0196Felicio D. Roberts  
for

# INTERNATIONAL SEARCH REPORT

International application No.

PCT/US05/35910

## Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:  
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☒ Claims Nos.: 19  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
  2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of any additional fees.
  3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
  4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
- Remark on Protest
- |                          |   |
|--------------------------|---|
| <input type="checkbox"/> | The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.   |
| <input type="checkbox"/> | The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation. |
| <input type="checkbox"/> | No protest accompanied the payment of additional search fees.   |

# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US05/35910

Continuation of B. FIELDS SEARCHED Item 3:

WEST and STN Medline

method, treatment, antibodies, \$TNF\$ and anti-viral or antibody or RSV or anti-viral therapy)

CORRECTED VERSION

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(30) Priority Data:  
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